A Color Atlas of Morphologic Hematology

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WITH A GUIDE TO CLINICAL INTERPRETATION

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PREFACE

The purpose of this *Atlas* is to furnish a guide for reference in the study of films of peripheral blood stained with Wright s stain The description of the blood in a variety of clinical conditions is considered from a diagnostic point of view The maturation of the blood cells is described and shown in the diagrams and plates so that immature or abnormal forms may be recognized in the peripheral blood or bone marrow It is emphasized that these diagrams are oversimplified for use as working hypotheses by those who are concerned with diagnosis and treatment No attempt is made to discuss in detail the many theories regarding the origin of the cells or to show in the diagrams other possible concepts The terminology used or the theories of cell origin are not as important as the recognition of the significance of the findings from a diagnostic prognostic and therapeutic point of view The morphology of cells of the bone marrow is not discussed separately since cells of the bone marrow may appear in the peripheral blood in certain physiologic or pathologic conditions The details of quantitative methods special tests as well as physiologic and pathologic aspects of the diseases involving red cells white cells and platelets are given in *A syllabus of laboratory examinations in clinical diagnosis* [116] which is a companion book of this *Atlas*

The author is indebted to the late Dr George R Minot whose guidance and enthusiasm were enjoyed for many years The constant encouragement support and guidance of Dr William B Castle and Dr Thomas Hale Ham have made progress in this field possible The author wishes to acknowledge also the assistance and counsel given by Dr Frederic Parker Jr and Dr Henry Jackson Jr not only in the preparation of this volume but in the study of the morphology of the blood and the pathology of many unusual cases over many years

The value of this book is largely due to the excellent drawings by Etta Piotti whose untimely death occurred since her work for this volume was completed

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1 *DETECTION OF ABNORMALITIES OF FORMED ELEMENTS BY BRIEF SCREENING EXAMINATION* A brief but critical screening examination of the blood film stained with Wright's stain is an essential part of the complete examination of each patient Abnormalities of the red cells the white cells and the platelets can be detected rapidly by inspection If abnormalities are apparent other examinations are indicated such as red cell count determination of the red cell indices of Wintrobe reticulocyte count white cell count platelet count bone marrow aspirations and other special tests discussed in the *Syllabus* [116] The variation of the red cells from normal is observed in respect to their size shape and hemoglobin content and the presence of abnormal forms as discussed in Sec 5 and shown in Plate I is noted It is usually possible to estimate whether the white cell count is normal increased or decreased An important observation is the deviation from the normal proportion of white cells as shown by neutrophilia lymphocytosis monocytosis eosinophilia neutropenia or lymphopenia The finding of immature cells or of cells not normally seen in the peripheral blood is of great importance Slight variations from the normal in the number of platelets observed in a blood film are of little clinical significance However the absence of platelets or a definite decrease in their number may indicate a primary or secondary thrombocytopenia The presence of large or abnormal platelets may indicate a fundamental disturbance of the bone marrow

2 *EVALUATION OF THE ACTIVITY OF THE BONE MARROW FROM EXAMINATION OF THE PERIPHERAL BLOOD* The degree of activity of the bone marrow in the production of formed elements may be evident in the film of peripheral blood For example all the formed elements may be decreased in the peripheral blood this condition is described by the term pancytopenia The presence of moderate numbers of polychromatophilic macrocytes in a blood film showing normochromic or hypochromic red cells suggests the recent occurrence of blood loss and a physiologic response of the bone marrow The occurrence of polychromatophilic cells in large numbers in chronic anemia with icterus with or without spheroidal red cells strongly suggests a hemolytic anemia Examination of the blood film is of value in determining the response or lack of response of pernicious anemia and the related macrocytic anemias to vitamin B_{12} (liver extract) or of hypochromic iron-deficient anemia to the administration of iron The presence of polychromatophilic cells or better the actual number of reticulocytes indicates the degree of erythropoietic activity of the bone marrow

3 *CONFIRMATION OF A DIAGNOSIS OR SUPPLEMENTING OTHER DATA* There are many instances in clinical medicine in which data from the blood film aid in confirming a diagnosis or supplement other data available to the physician Thus the identification of the abnormal cells characteristic of infectious mononucleosis aids materially in distinguishing it from the fatal disease leukemia Also the blood film is essential for the establishment of a clinical diagnosis of malaria Oroya fever and certain other parasitic diseases Most pyogenic infections are associated with leukocytosis and neutrophilia whereas leukopenia or neutropenia may occur in such infections as typhoid fever brucellosis and virus infections The presence of eosinophilia may suggest a diagnosis of trichinosis

2 PREPARATION OF BLOOD FILMS

A thin well stained film of blood is essential for the critical evaluation of the blood cells The blood film should be thin enough that the red cells do not overlap and that the white cells are

spread out for observation of details of the nuclear structure, color of the cytoplasm type of granules and relative size of the cell

For successful preparation of films of blood, the slides or coverglasses must be washed free from acid and alkali which alter the staining reaction and free from grease which interferes with uniform spreading of the blood film For the most accurate results the differential count of white cells should be made on a stained film prepared on a coverglass (No 0 or No 1 ⅞ inch square) In such films, the blood spreads by capillary action and the dispersion of the leukocytes is that of a chance distribution [13 116] In slide preparations the distribution of leukocytes is not uniform since the large red and white cells accumulate in increased numbers along the edge and at the end of the blood film Slide preparations are preferred for the work in parasitology, where a thick drop and a thin film may be prepared on the same slide

1 *SOURCE OF THE SPECIMEN* When a sample of venous blood is taken for blood counts a small drop of blood from the tip of the needle is placed on a coverglass and covered with another coverglass held in such a position as to form with the first an eight pointed star As soon as the drop of blood has spread over the surface the coverglasses are separated by sliding the upper one off of the lower one, holding them in the same plane in which they are lying Coverglasses should never be separated by lifting Preparations are air dried and placed vertically in a box with slits or partitions

For some purposes capillary blood is sufficient or preferred A sterile instrument for puncturing the lobe of the ear or the tip of the finger should always be used and should always be resterilized before being used again The Fenwal lancets, hemolets or a No 11 Bard Parker blade have been found to be satisfactory Obtaining the blood sample from the ear is preferred to obtaining it from the finger because it causes less discomfort to the patient and is less likely to lead to infection It has been found however that the white cell count in blood from the ear is normally slightly greater than in that from the finger [59 177] and that large white cells may be present in greater numbers This may be a distinct advantage in the study of the blood in bacterial endocarditis, infectious mononucleosis, leukopenia and leukemia Blood samples from the finger give results comparable to those in the venous sample The red cell counts and hemoglobin are essentially the same in the samples from the ear as in those from the finger and the vein In children blood is usually collected from the finger if it is not possible or necessary to obtain a venous sample In infants, the puncture is usually made in the heel

In an emergency, blood films may be made from samples of venous blood containing anticoagulants but they are not satisfactory for the morphologic study of white cells since white cells change in their staining characteristics develop vacuoles, phagocytose crystals of oxalate and degenerate gradually on standing As a result, the nucleus of the lymphocyte may appear to be dividing the nucleus of the monocyte often appears as a clover leaf and the lobes of the neutrophil are often separated into distinct parts

2 *PRINCIPLES AND METHODS OF STAINING THE BLOOD FILM*

WRIGHT S STAIN Wright s stain, a common modification of the Romanowsky stain is a combination of methylene blue methylene azure and eosin As stated by Conn [49], these compound dyes act on the protoplasm somewhat as follows certain parts of the cell have an affinity for the neutral stain and take it up as such others having an affinity for the basic dye, break up the neutral stain so as to obtain the basic portion of it or, if dissociation has taken place take up the basic ion directly while other parts of the cell with an affinity for acid dyes similarly combine with the acid portion of the stain The nucleus of the cells or especially the chromatin within the nuclei is assumed to be acid in character (owing largely to the constituent nucleic acid), has an affinity for a basic dye (methylene blue) and is termed basophilic Neutral material is stained violet in color and is termed neutrophilic Basic material is stained by an acid dye (eosin) and is termed eosinophilic Azurophilic as the name implies indicates an affinity for methylene azure which is red purple

Wright s stain certified by the Commission on Staining [49] may be purchased in solution or

in powdered form. The solution is prepared by dissolving 0.1 gm of powder in 60 ml of absolute methyl alcohol (acetone-free and chemically pure). The stain should be filtered when made and whenever samples are taken from the stock solution. Care should be taken to keep the reagent bottles stoppered and to avoid contamination with water or acid.

The buffer solution for use in diluting the Wright's stain should have a pH of 6.4 to 6.8. A phosphate buffer solution with pH 6.7 may be prepared by mixing 43.5 ml of M/15 anhydrous secondary sodium phosphate (Na_2HPO_4 9.47 gm/lit) and 56.5 ml of M/15 primary potassium phosphate (KH_2PO_4 9.08 gm/lit).

The coverglasses preferably with fresh but dry blood films are placed in a horizontal position on a flat support or a rubber stopper and covered with Wright's stain. After remaining for 3 min the stain is diluted with an approximately equal volume of buffer or tap water and allowed to stain for another 3 min. The pH of the diluting fluid should be 6.4 to 6.8; distilled water is often too acid. The stain is then floated off by holding the coverglass in a horizontal plane under the running tap water for a short time. The preparation is then dried thoroughly by careful blotting with filter paper. When dry, any extra stain on the back of the coverglass is removed with gauze moistened in alcohol. The dry coverglass preparations are mounted with the blood film face down on a clean glass slide on which has been placed a drop of isobutyl methacrylate dissolved in xylol. The mounting fluid should be in the same pH range (6.4 to 6.8) as the stain. When an acid mounting fluid is used the staining of the cells on the films deteriorates with time. Enough mounting fluid should be used to spread completely over the surface and air bubbles should be avoided. For satisfactory staining and preservation of blood films contamination with acid or acid fumes should be avoided throughout the procedure. Evaporation of the alcohol of the stain during the procedure causes undesirable precipitate and often produces a muddy background.

BRILLIANT CRESYL BLUE FOR RETICULOCYTES. Brilliant cresyl blue is used as a vital stain to precipitate the basophilic substance of the red cells which appears polychromatophilic when stained only with Wright's stain. The cells containing this precipitated basophilic substance are called reticulocytes. Counts may be performed on fresh preparations or permanent preparations counterstained with Wright's stain. Whatever method is chosen for a particular study should be used throughout the investigation to assure comparable results. Prolonged contact of the dye with the cells will give slightly higher values for reticulocytes than a brief exposure. The advantage of the permanent preparation can be readily appreciated since the counts do not have to be made immediately and the slides may be filed for reference. In reticulocyte preparations the basophilic substance of the white cells is also precipitated in the cytoplasm so that differentiation of the white cells is sometimes difficult. Such preparations should be used for a differential white cell count only when plain Wright's stained films are not available.

For the permanent preparations an alcoholic solution of brilliant cresyl blue is used. It is made by dissolving 0.5 gm of brilliant cresyl blue in 100 ml of 95 percent ethyl alcohol. This is a saturated solution and should be filtered when made up and each time aliquots are taken from the stock solution for the preparation of cresyl blue coverglasses. Evaporation of the stock solution will cause precipitation of the stain.

Preparation of the cresyl blue coverglasses requires the use of coverglasses that have been carefully cleaned and dried. Coverglasses are usually left for several hours in cleaning solution (potassium dichromate and sulfuric acid) or in soap and water. They should then be rinsed thoroughly until the rinse water gives a neutral reaction to litmus. The coverglasses are then stored in alcohol until dried for use. The coverglasses are placed on a flat surface and just enough (1 or 2 drops) of the alcoholic solution of brilliant cresyl blue is added to cover the surface of each coverglass. These are covered with a glass beaker or cover to reduce the rate of evaporation of the alcohol. When dry the stained surface appears gray. The coverglass is polished until a purple color is obtained by rubbing lightly stain side down on a smooth or glazed paper. These prepared coverslips are stored stained surfaces together until needed. A convenient rack may be made by cut

ting slits in the cover of a cardboard box in which the corners of the coverglasses may be placed

The reticulocytes are stained by placing a drop of blood on the stained surface of one of these previously prepared coverglasses A second cover glass stained surface down, is placed crosswise on the drop of blood, making an eight pointed star When the blood has spread over the entire surface *by capillarity, not pressure, the two cov* erglasses should be separated by sliding them apart promptly and in the same plane in which they are lying When the films are dry, they should be counterstained with Wright s stain

3 *EXAMINATION OF THE QUALITY OF THE STAINED BLOOD FILM* A general survey of the blood film should be made using the high dry objective of the microscope, to judge the quality of the film In most fields examined, the red cells and white cells should be clearly separated should show no artifacts such as vacuoles in the red cells and should not show significant *amounts of precipitate The staining should be* uniform throughout The stain should be alkaline enough to stain clearly the platelets polychromatophilic cells granules in the neutrophilic series stippled cells, and the nuclei of the cells and acid enough to bring out the contrast between the nuclei and the cytoplasm of neutrophils

The optimum pH is about 6 4 to 6 8 for the buffer solution used in diluting the Wright s stain on the film Excessive red staining or red areas are due to an acid reaction This might be caused by acid fumes while staining coverglasses that were not thoroughly rinsed, or areas that were still moist when the coverglass was mounted Excessive blue staining may be due to alkali to excessive thickness of the film, or to evaporation of the alcoholic stain and drying of the film In thick films when the red cells are piled up, the cyto*plasm of the white cells will* be condensed about the nucleus so that structure cannot be observed and the granules are not distinct enough to be identified

The distribution of the cells should be uniform without a concentration of cells at one end or around the periphery This condition is difficult to obtain in the slide preparation Precipitate on the film interferes with the recognition of platelets and reticulocytes and may be avoided by floating off the precipitate formed in staining by thorough washing of the films and by filtering the stain frequently The presence of small bub*blelike deformities of the surface of the red cells* is an artifact indicating that the film was mounted before being thoroughly dried The mounting medium preferably methacrylate should spread beneath the entire film since air pockets interfere with clear definition of the cells If the stained blood film is unsatisfactory, it is a waste of time to examine it in detail since the results are unreliable and may be seriously misleading Another blood film should be prepared

3 PREPARATION OF THE COLOR PLATES

1 *USE OF WRIGHT S STAIN IN THIS AT*LAS The films of peripheral blood reproduced in this *Color atlas* were stained with Wright s stain Although this is a limited method of study, it is of practical value since Wright s stain is widely used in clinical medicine Accordingly the color values and descriptions in this book are limited to those obtained by using Wright s stain and brilliant cresyl blue *The structure of the cells* as described results in part from fixation by methyl alcohol contained in Wright s stain The color of *the stained* cells is an expression of the affinity of the cellular elements for the acid basic or neutral portions of this stain Other methods of staining such as supravital staining the peroxidase stain and histochemical methods, are of value in special instances [116])

2 *PREPARATION AND USE OF THE COLOR PLATES* The cells in the color plates (Plates I to XVI) were drawn using a camera lucida and painted in water color as they were observed through the microscope with a magnifica*tion of 1200* Each plate represents a composite of two or three oil immersion fields from blood films of patients with proved diagnoses In these plates will be found the cells that occur in peripheral blood in the normal subject as well as most of the progenitors of blood cells The immature

forms that normally are found in bone marrow or other tissues may occur in the peripheral blood in abnormal states Thus the identification of cells in these plates of peripheral blood will aid in performing differential cell counts of aspirations of bone marrow The plates are limited in number and represent conditions in which the film of blood is of definite diagnostic value Emphasis is placed on the interpretation of the cells found in these films and on the differential diagno is of the conditions in which related abnormalities may be found

4 GENERAL CHARACTERISTICS OF FORMED ELEMENTS OF BLOOD STAINED WITH WRIGHT'S STAIN

1 *CHARACTERISTICS OF THE CELL* The formed elements of the blood include the nucleated red cells and white cells the nonnucleated mature red cells and the platelets The cytoplasm enclosed in cell membrane may appear clear or may contain granules rods or foreign particles that have been phagocyto ed The nucleus of white cells and of nucleated red cells contains a network of chromatin material and in the primitive cells one or more nucleoli may be present within the nucleus With Wright s stain the nucleolus appears as a circumscribed area about 2 to 3 μ in diameter that is less densely stained than the surrounding nucleus In films stained with brilliant cresyl blue the nucleolus appears as a light blue area Nucleoli should be distinguished from dark staining clumps of chromatin and from vacuoles which may appear as clear colorless areas of variable size and number in either the nucleu or the cytoplasm of the cell Films that have been stored for years sometimes show degenerative changes or clear areas in the nuclear chromatin that are not nucleoli In films of bone marrow aspirations or in the peripheral blood in the leukemias nuclear division or mitosis may be seen

2 *IDENTIFICATION OF CELLS* For study and identification of the cells in a film of blood it is essential to examine the preparation with oil immersion magnification (1000 to 1200) and adequate illumination It is necessary not only to see the cells but to see the inside of the cells to study the color of the granules and the details of the structure of the nucleus The variation in types of blood cells is great Cells may be identified as belonging to the red-cell series the white cell series or the thrombocytic series Further classification of the white cells may be made by grouping them into the granulocytic lymphocytic monocytic plasmacytic or histiocytic series To understand the different cells in each series the several stages of development of the cell must be appreciated (see Figs 4 5, 6 7 9) These stages may be seen in aspirations of bone marrow and also in the peripheral blood in certain infections and in anemia and leukemia The degree of immaturity of the blood cells seen in the peripheral blood is a rough index of the physiologic response of the bone marrow or other tissues or may indicate abnormal growth as in neoplastic diseases The appearance of abortive types of cells is also a sign of abnormality that may have a serious prognosis Such cells may be present in a terminal illness in overwhelming sepsis or after prolonged irradiation

An accurate report of the blood findings on the part of the technologist is important An understanding of these abnormal cells and their significance is equally important for the physician in order that he may interpret the physiologic and pathologic significance of the laboratory data

If a cell is difficult to classify it should be compared with cells that can be recognized for the structure of the nucleus the color and structure of the cytoplasm and the type and color of the granules If a cell cannot be recognized as belonging to any of the established series it should be recorded as an abnormal cell and described in detail with a drawing

Cell development can be described in a systematic manner since the morphologic changes are similar in some respects for each series of cells described here Some generalizations will be helpful in studying the cells although there are many exceptions to all the rules that can be established Differentiation of the type and age of the cells

must be determined by a consideration of many factors Some of these factors are size, structure of the nucleus color of nuclear chromatin, presence of nucleoli character and color of the cytoplasm relative proportion of nucleus and cytoplasm granulation, and color of the granules No one factor can be considered alone although the structure of the nucleus is probably the most valuable single guide

(a) Relative Size of Cells In general immature cells are larger than mature cells The stem cell is definitely larger than the other blasts of any series (Figs 1, 4 5 6, 7, and 9) Other examples may be seen by comparing the proerythroblast with the normoblast, the polychromatophilic cell with the mature red cell (Fig 1), and the myelocyte with the mature granulocyte (Fig 4) Abnormal eosinophils in infectious states are usually *larger than the normal eosinophil In infections* the myelocytes and band forms are larger than the neutrophils and the neutrophils may be larger than those in normal blood The mature small lymphocyte is the smallest cell of the white cell series The plasmablast and the young plasma cell are larger than the mature plasma cell although the mature cell may be large in the leukemic states

(b) Relative Size of Nucleus and Cytoplasm The nucleus in the immature cell takes up a larger proportion of the cell than it does in the mature cell Examples of this are especially striking in *the granulocytic series (Fig 4) In the* lymphocytic series (Fig 5) this generalization holds except for the small lymphocyte in which the nucleus occupies most of the cell

(c) Nuclear Structure as a Distinguishing Characteristic The characteristics of the nuclear structure are illustrated diagrammatically in Figs 1, 4 5 6, 7 8 9 and in more exact detail in the color plates (I through XVI) These details are realized most effectively if the observer reproduces the cells on paper either with pencil or with colored crayons

In general the young cells have a uniformly granular structure *of the chromatin of the nucleus* while the mature forms of each series are characterized by a heavy condensation of chromatin as is seen in the small lymphocyte, plasma cell neutrophil and normoblast However the monocyte and histiocyte are definitely exceptions *to this generalization The color of the nuclei of* the mature cells in general is blue purple and is more intense than the red purple of the blast nuclei Nucleoli are present in the youngest *forms It is of utmost importance to differentiate the* blast from other cells by careful observation of the type of the nucleus The series to which a blast cell belongs is determined in part by the size of the cell the color of the cytoplasm and the structure of the nucleus as compared with other blasts and in part by the presence of other cells of the same series ("by the company they keep") Blasts from more than one series may be present, as for example granulocytic and erythrocytic series See also pp 26, 35, 39, 40 Plates V, VII VIII XI

(d) Color of the Cytoplasm as a Differential Criterion *The stem cell the proerythro*blast and all the blasts of the white cell series are characterized by the deep basophilia of the cytoplasm although the shade and character of this deep blue varies somewhat in the different series The proerythroblast has a gray blue cytoplasm somewhat similar to that of the monoblast The cytoplasm of the myeloblast is usually rather a clear deep blue but is more abundant than that of the lymphoblast

The change from the deep basophilia of the proerythroblast to the hemoglobin containing cytoplasm of the normoblast and the mature eryth*rocyte may be seen diagrammatically in Fig 1* and in Plate I In the intermediate stages both elements — the basophilic substance and the hemoglobin — are factors in producing the polychromatophilia of the cytoplasm the relative amount of each substance determining the intensity of the color As the cell matures basophilic substance decreases and hemoglobin increases

The disappearance of the basophilic substance as the cell matures is illustrated in the granulocytic series by comparison of the cytoplasm of myelocyte A with that of myelocyte C (Plate XI)

In the lymphocytic series the mature small lym*phocyte and the large lymphocyte have the light*est blue cytoplasm of any of the blood cells However the young lymphocyte has practically as blue a cytoplasm as the lymphoblast Some small lymphocytes do have deep blue cytoplasm which

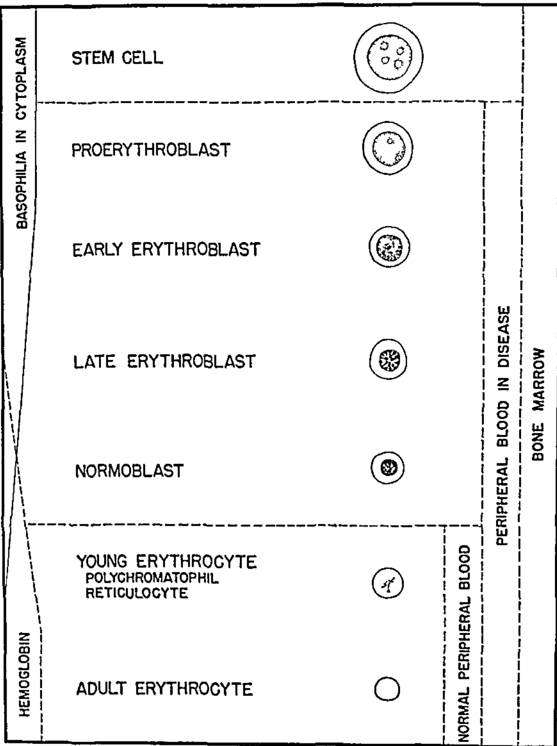


FIG 1 Diagram of the maturation of the erythrocytic series

may be due to a crowded area in the film with resulting condensation of the cytoplasm about the nucleus or they may be young forms according to the studies of Wiseman [308] If the films are thick it is difficult to tell the lymphocyte from the monocyte because the details of the cytoplasm cannot be seen The mature plasma cell has a deep blue cytoplasm which is usually less intense than the blast of the same series.

(e) COLOR VALUES Use of the compound, polychromatic Romanowsky stains such as Wright s stain [49], affords the advantage of staining the acidophilic and basophilic elements at the same time This actually means that there is a gradation of staining reaction from the deep blue (acid in reaction but basophilic ' in staining) of the cytoplasm of the blast to the red (basic in reaction but acidophilic in staining) of the mature red cell This gradation in color value applies to the nucleus to the cytoplasm, and to the granules and may be more clearly understood from the systematic color scheme shown in Fig 2 In this chart the color relations of the cellular elements of the blood (nucleus, cytoplasm and granules) are shown graphically in a color circle Practically all of the colors developed in the cells by the Wright s stain are within one sector of this circle or between the blue and the red This produces a harmony in the colors of the stained blood cells that is pleasing to the eye It also shows why it is difficult to describe the delicate changes and slight differences in color The chart expresses only color value the intensity of color (pink to red or light blue to deep blue) is not indicated The points indicated in the center and on the periphery of the circle are the colors at their fullest intensity The primary colors are indicated by the solid lines and the secondary colors by the broken lines

5 PLATE I THE RED CELLS AND THE ERYTHROCYTIC SERIES (Fig 1)

1 TERMINOLOGY OF THE NUCLEATED FORMS OF THE ERYTHROCYTIC SERIES Before a study of abnormal bloods can be undertaken the description and terminology of the normal and abnormal forms of red cells must be understood In the description of the nucleated forms the characteristics of the nuclear chromatin serve as criteria for classification, since the size of the cell and the color of the cytoplasm are so variable.

All investigators are agreed that the nucleated red cells seen in the blood and in the bone marrow aspirations in pernicious anemia are abnormally large and that the bone marrow shows an increase in proportion of cells with deeply basophilic cytoplasm These cells are called megaloblasts by many authors Many of these cells are strikingly atypical showing clumped or reticular structure of the nucleus while the cytoplasm is still deeply basophilic Normally this type of nuclear structure occurs with a more mature cell as evidenced by cytoplasm that is less basophilic There is no evidence that these cells have an origin different from that of normal red cells although their maturation is abnormal

Many different names have been applied to the cells of the erythrocytic series The following list gives the names used in this *Atlas*, in italics, together with the terms employed by other authors —*stem cell* haemoblast [224] lymphoidocyte [94 274] hemocytoblast [56 139, 185, 301] erythrogone [67] *proerythroblast* [54 94, 139, 170 224 280] megaloblast [75 126], normoblast A [67], pronormoblast [56 80, 150 306] rubriblast [48] *early erythroblast* [54, 75] erythroblast [126] basophilic erythroblast [54 94 185 280] basophilic normoblast [56 80, 150 170 306] early normoblast [139 301] prorubricyte [48], normoblast B [6] *late erythroblast* [54 75] polychromatic erythroblast [54 94 185 280] polychromatic normoblast [56 80 150 170, 306] pronormoblast [126] intermediate normoblast [139 301] *normoblast* [54 75 126] acidophilic erythroblast [54] eosinophilic erythroblast [280] orthochromatic erythroblast [94 224] orthochromatic normoblast [80 150 306], pyknotic normoblast [56], late normoblast [139 301] normoblast C [67], metarubricyte [48] *young erythrocyte (reticulocyte or polychromatophil)* *adult erythrocyte* rubricyte [48]

Fig 2 Graphic representation of the color value of the nucleus, cytoplasm and granules of blood cells stained with Wright's stain

2 *DESCRIPTION OF NORMAL AND ABNORMAL CELLS OF ERYTHROCYTIC SERIES (PLATE 1)* The cells are described in order from the mature erythrocyte to the least mature form, the proerythroblast The figures in italics refer to the cells shown in the plate

(*a*) ERYTHROCYTE (mature red cell, *1*) The diameter of the normal red cell is between 5 and 9 μ with a mean of 7 2 to 7 8 μ in normal individuals The diameter of the abnormal red cell may vary from 2 to 14 μ The color of the cell is a yellow red owing to staining of hemoglobin The pale central area results from the thinness of the layer of hemoglobin due to the biconcave shape of the normal red cell No nucleus or granules are present in the normal red cell

(*b*) POLYCHROMATOPHILIC ERYTHROCYTE (immature red cell *2*) This cell is usually larger than the adult cell of the same blood sample The polychromatophilia is evidenced by the blue purple color of the cell No nucleus or granules are normally present but nucleated red cells may also show polychromasia of the cytoplasm

Reticulocyte (immature red cell *7a 7b 7c*) This cell is the same stage as the polychromatophilic erythrocyte but is vitally stained with brilliant cresyl blue (pp 3 14) so that it can be recognized and counted more easily than polychromatophilic cells The basophilic material in the cell is precipitated by the cresyl blue as a heavy network of blue substance in the younger forms (*7a*) a moderate amount in the intermediate forms (*7b*) or fine scattered reticulum in the older forms (*7c*) Accordingly there is a quantitative relation between the amount of reticulum in the cell and the age of the cell [123] There should be no polychromatophilic cells remaining in a film stained for reticulocytes since all of the diffuse polychromatophilic material should appear as precipitated reticulum

(*c*) NORMOBLAST (*3*) The normoblast may vary in diameter from a normal range of 6 or 7 μ up to 12 to 14 μ in pernicious anemia The cytoplasm may be acidophilic or polychromatophilic The nucleus is a small condensed mass of chromatin usually deep purple Very little structure is evident in this compact nucleus In pathologic conditions the nucleus may be divided into two three or more portions (*3e*) In the blood film stained with brilliant cresyl blue reticulum may be very dense (*3b*) in cells that would show marked polychromatophilia with Wright s stain alone

(*d*) LATE ERYTHROBLAST (*4*) The erythroblast is larger than the erythrocyte or normoblast The nuclear chromatin is broken up into segments sometimes radially arranged like the spokes of a wheel The cytoplasm at this stage is usually polychromatophilic For this reason this cell is termed polychromatophilic erythroblast ' by some hematologists

(*e*) EARLY ERYTHROBLAST (*5*) The chromatin of the nucleus of the early erythroblast is broken up into fine clumps It is not as granular as in the proerythroblast but is more finely divided than in the late erythroblast The cytoplasm is basophilic

(*f*) PROERYTHROBLAST (*6*) The proerythroblast is the youngest form of the erythrocytic series that can be distinguished from the blasts of the white cell series It is usually larger than the normoblast or erythroblast The cytoplasm is a deep blue (basophilic) with a little more purple than in the other blasts The nucleus is made up of fine uniformly granular chromatin is purple in color and is definitely round in shape Nucleoli are usually present

(*g*) STEM CELL The stem cell is larger than any other blast form (see Plate XV *8*) The finely granular purple nucleus is less intense in color than the deeply basophilic cytoplasm More than one nucleolus may be present in this primitive cell The stem cell may be seen in the peripheral blood in leukemia in association with other blast forms or in stem cell leukemia in which the type of cell is primitive and its series cannot be identified

(*h*) NUCLEATED RED CELLS IN THE PERIPHERAL BLOOD Under normal conditions nucleated red cells are found only in the circulating blood of the fetus and newborn infants Nucleated red cells in the peripheral blood in the adult may occur as a physiologic response to severe anoxia whether due to anemia from hemorrhage hemolytic anemia, nutritional or pernicious anemia or impairment of pulmonary or cardiac functions The presence of nucleated red cells in the periph

eral blood when no evidence of anoxia exists (that is when the hemoglobin exceeds 10 gm/100 ml of blood and when no cardiac or pulmonary disease is present) should suggest a myelophthisic anemia Replacement of the bone marrow as a result of carcinoma lymphoma leukemia or fibrosis may cause a myelophthisic anemia

The pathologic forms of nucleated red cells that are seen in the peripheral blood in the macrocytic anemias associated with Addisonian pernicious anemia nutritional deficiency sprue pernicious anemia of pregnancy and diphyllobothrium latum infestation are collectively called megaloblasts by many authors These pathologic forms occur in all stages of the red cell development and include abnormal normoblasts (*3a*) abnormal late erythroblasts (*4a*) abnormal early erythroblasts (*5a*) abnormal proerythroblasts (*6a*) and cells showing mitosis (*13*) Many hematologists believe that these pathologic forms of the erythrocytic series show a qualitative change as a result of a deficiency of an essential maturation factor (vitamin B folic acid or citrovorum factor) and do not represent a series of cells of separate origin These pathologic features are macrocytosis abnormal reticular structure of the nuclear chromatin dissynchronism of the maturation of the cytoplasm and the nucleus and mitosis Similar abnormalities of size and chromatin structure can also be seen in the circulating granulocytes in these conditions These changes in red cells in white cells and also in megakaryocytes are much more striking in the bone marrow than in the peripheral blood and serve as important diagnostic aids

The normoblasts in the peripheral blood in iron-deficiency anemia are small and have a scanty amount of basophilic or polychromatophilic cytoplasm The maturation of the cytoplasm appears to be slower than the maturation of the compact nucleus of the cell These iron deficient normoblasts are even more striking in the bone marrow

In hemolytic anemia the nucleated red cells in the peripheral blood are normocytic or macrocytic they have a normal proportion of the cytoplasmic and nuclear material and normal structure of nuclear chromatin Normoblasts with two nuclei (*3e*) with Howell Jolly bodies (*3d*) with stippling (*3c*) with reticulum (*3b*) and with various other abnormal formations may occur in the hemolytic anemias These abnormalities are even more striking after splenectomy In the more severe hemolytic reactions erythroblasts and rarely proerythroblasts may be present in the peripheral blood

In the myelophthisic anemias the nucleated red cells are usually normal in size but may be abortive in form often having multilobed nuclei or nuclear fragments in the cytoplasm.

(1) STIPPLING GRANULES NUCLEAR REMNANTS AND INCLUSION BODIES IN RED CELLS

Stippled red cells (punctate basophilia) Stippling denotes the presence of round blue purple granules of variable size scattered throughout the mature polychromatophilic (*8*) or nucleated (*3c*) red blood cell Stippled red cells may be seen in films prepared with Wright s stain alone They are also evident in cresyl blue preparations but should not be confused with the blue network of the reticulocytes

Although stippling is a regular characteristic o[illegible] lead poisoning it may be seen in normal [illegible] [92] in leukemia and in practically all the [illegible] mias especially in myelophthisic anemia an[illegible] Cooley s anemia and Cooley s trait

Siderocytes (*10* and *10a*) Nucleated and non nucleated forms of the red cell with siderotic granules which contain free iron have been termed siderocytes [112] It is important to recognize and distinguish them from the punctate basophilia of stippled cells and from Howell Jolly bodies When stained with Wright s stain siderotic granules may appear singly as small blue purple bodies or occur in pairs or tetrads near the periphery of the cell In contrast to the iron contained in hemoglobin which does not stain the free iron of the siderotic granule stains greenish blue with the Prussian blue reaction or with acid iron stain [111 179] A dried film of blood is first fixed in methyl alcohol for 10 min and then is dried It is then immersed for approximately 10 min in a freshly prepared mixture of equal parts of aqueous potassium ferrocyanide (2 gm/100 ml) and aqueous hydrochloric acid (1 percent by volume) The slide is washed dried and counter stained with Biebrich scarlet safranin or 0 1 percent aqueous basic fuchsin Finally the prep-

aration is washed mounted and examined with the oil immersion objective

Siderocytes have been seen in the embryo and in nucleated red cells of the bone marrow of the adult but not in the mature erythrocyte of the normal adult [78] Large numbers may be seen in hemolytic anemia, especially after splenectomy [179 225]

Howell Jolly bodies (nuclear remnant 9) Howell Jolly bodies are small (1 to 3 μ) purple blue round bodies which may occur singly or in multiples in the red cell They are stained the same as the nuclei of normoblasts and are considered to be remnants of the nucleus [202] The cell containing Howell Jolly bodies (9) should be compared with the normoblast with Howell Jolly bodies (3d) In the normoblast the nucleus apparently is being extruded while two fragments of the nucleus — the size of Howell Jolly bodies — remain The diameters of these bodies may vary from that of a small nucleus down to 0 5 μ Howell Jolly bodies are seen in severe anemias such as pernicious anemia Cooley s anemia erythroblastosis and sickle cell anemia They are increased in the blood of normal persons following splenectomy [168] and appear to be found characteristically in patients with atrophy of the spleen [28] in a variety of conditions including sprue and nontropical sprue

Cabot ring forms (14) Red cells in certain anemias occasionally show a delicate violet threadlike structure in the form of a ring or twisted ring or figure of eight Cabot [35] originally described these structures and considered them to be remnants of the nuclear membrane Schleicher [255], however has presented evidence that the rings are artifacts due to formation of denatured protein The rare occurrence of these rings has made study of them difficult

Refractive granule of red blood cell (11) A refractive body about 0 5 μ in diameter occurring in less than 1 percent of normal red cells was described by Isaacs [136] This granule which occurs singly appears black in one focal plane of the microscope and clear and colorless (refractive) in another It does not take any ordinary stains and is of unknown origin and composition The occurrence of granules is similar to that of the reticulocytes but red cells containing granules are considered to represent a more mature or intermediate stage between the reticulocyte and the mature erythrocyte

Heinz Ehrlich bodies (refractive bodies 12) Refractive bodies in red cells described by Heinz [125] and by Ehrlich [84] are seen in wet preparations and are not stained by Romanowsky stains They appear in the red cells as round refractile inclusion bodies with Brownian movement varying in diameter from 0 5 to 1 μ They appear to be newly formed particles containing protein (of unknown origin) and occur in the course of irreversible injury to the red cell by certain toxic agents [95 298] In contrast to the refractive granule of Isaacs the Heinz-Ehrlich bodies are associated with hemolytic anemias from such compounds as phenylhydrazine erythrol tetranitrate and the sulfonamides An extensive study of other compounds that produce Heinz Ehrlich bodies has been made by Fertman and Fertman [96] They can be stained vitally by employing a half saturated solution of methyl violet or crystal violet in saline [299] One drop of this solution and one drop of blood are placed on a slide covered with a coverglass and observed under high magnification (× 1000) The granules appear as blue violet particles often near the periphery of the cell

(j) ABNORMAL SHAPES OF RED CELLS The terms *anisocytosis* (abnormal variation in size) and *poikilocytosis* (abnormal variation in shape) have been used too loosely in describing the red cells in pathologic conditions Instead of using these terms it is of greater diagnostic value to observe and describe the particular abnormal forms and associate them with a specific disease entity For example the presence of polychromatophilic macrocytes usually means active regeneration of blood which may be due to hemolytic anemia acute blood loss or response to therapy The presence of definite sickled forms (24) is diagnostic of the sickle cell phenomenon but the phenomenon should be confirmed by a specific test

Spheroidal red cells (spherocytes 19) The spheroidal red cell is a small deeply stained cell without central pallor in which the hemoglobin appears increased Since the volume of the spherocyte is approximately normal but its diameter is

less than normal it is apparent geometrically that its thickness is increased Microscopically the cells appear hyperchromic not only because of this increased thickness but also because of their increased corpuscular hemoglobin concentration (MCHC) Spheroidal red cells have increased osmotic and mechanical fragility and they occur in congenital hemolytic jaundice (hereditary spherocytosis) in other hemolytic anemias such as acquired hemolytic anemia following thermal burns [260] and in certain drug intoxications [87]

Oval cells (elliptic cells) Oval macrocytes (*16*) are characteristic cells that are usually seen in the blood film of untreated pernicious anemia However oval cells (*17*) that are approximately normal in size are occasionally seen in increased numbers in the peripheral blood as an inherited Mendelian dominant characteristic [279] A comprehensive study of 86 cases in three interrelated families was made by Wyandt Bancroft and Winship [313] Only one of these cases showed striking anemia This patient undoubtedly had the oval-cell characteristic in the homozygous state while the other members had the factor as a heterozygote Lipton [176] has reported a patient who was homozygous for the elliptocytic trait and had a severe anemia Some individuals with oval cells have had by coincidence other blood dyscrasias such as pernicious anemia leukemia and hemolytic jaundice Oval cells do not sickle although some cases of ovalocytosis reported in the literature have been confused with sickle cell anemia

Pencil forms (*18*) The pencil form of red cells which may be confused with the oval cells is thinner in proportion to its length than the oval cell Pencil forms are usually hypochromic and are characteristically found in hypochromic anemia Cooley's anemia and Cooley's trait

Sickle cells (*24*) Sickled forms of red cells are sometimes but not always seen in the film of peripheral blood of a patient with sickle cell anemia. These may take the forms shown in Plate I (*24*) or they may have more rounded ends Those with rounded ends are permanently (irreversibly) sickled forms [259] Those with pointed ends or filaments seen in freshly sickled preparations, may not be evident in the untreated film of peripheral blood (See Plate VI)

Target cells (*26*) The target cell has created more interest and been described by more names (Mexican hat button form bull's-eye) than it deserves Because of its unique arrangement of hemoglobin it looks like a target that is there is a central area of hemoglobin (bull's-eye) surrounded by a clear ring without pigment outside of which is the pigmented border of the cell These cells are thin hypochromic cells which show an increased resistance to hemolysis in hypotonic solutions of sodium chloride [14 113] They are seen in increased numbers in severe hypochromic anemia sickle-cell anemia Cooley's anemia and trait liver disease and in patients with hemoglobin C disease and are often increased in number following splenectomy [268] Target cells may be produced in vitro by suspending normal red cells in plasma or serum rendered hypertonic either by addition of chemicals or by evaporation [293] It is possible to see target cells in certain areas of a film of normal blood their presence however may be due to abnormal drying of the film and should not be considered typical of the blood picture unless it is seen throughout the film.

Lunar forms (*20*) Lunar or half moon forms have been seen in acute hemolytic anemia (Plate VII) "Shadows" or ghosts of erythrocytes thin veils of membrane and crescent bodies — colorless veillike disks 40 to 50 μ in diameter with pink crescents in the margin — are found only rarely as a result of hemolysis [306]

Crenated red cells (*21*) Red cells are sometimes distorted owing to changes in surface These round cells have numerous regularly spaced points or spicules on the surface Such cells frequently occur in groups in certain areas of the film.

Burr cell (*22*) A poikilocyte with one to several large spiny projections on its periphery has been called a "burr" cell because of its resemblance to a burr This cell usually occurs singly and not in groups as do crenated cells Schwartz *et al* [257] found an increase of these cells in uremia carcinoma of the stomach and bleeding peptic ulcer

Acanthrocyte (*23*) The acanthrocyte is a deformed red cell that has numerous irregularly spaced and irregularly shaped pseudopods on its periphery giving the appearance of thorns [265]

These cells were originally reported by Bassen and Kornzweig [15] in a case of atypical retinitis pigmentosa

Irregular form (poikilocytes *25 27*) A variety of terms — such as pear shaped tailed or bizarre forms — are used to describe the irregular forms of cells seen in certain of the red cell diseases It is unknown by what mechanism these forms arise whether fragmentation of red cells budding of the cytoplasm to produce daughter cells or defective formation of cells [51, 77 248] Small round particles containing hemoglobin were called schistocytes by Ehrlich [86] It is known that fission of the red cells occurs as a result of heating [118 137] Tiny microcytes are often seen in the blood in hypochromic anemia pernicious anemia and Cooley s anemia The presence of a variety of these shapes and of microcytes is a characteristic feature and aids in the diagnosis of Cooley s anemia

3 *COUNTING RETICULOCYTES* In either a dry or a wet preparation the number of reticulocytes is determined by counting a total of 1000 red cells All red cells including nucleated forms that contain reticulum are counted as reticulocytes The results are usually expressed as a percentage of the total number of red cells or the absolute number of reticulocytes per unit volume may be calculated from the red cell count Because of the large number of cells in an oil immersion field it is helpful to cut down the area by inserting in one ocular of the microscope, a mask that has a central hole 3 to 5 mm in diameter Different areas where the film is thin and the cells are separated well stained and easily identified should be selected for counting

Counterstaining the vitally stained preparation with Wright s stain although not essential gives a sharp contrast between the blue of the reticulum and the red of the cell No polychromatophilic cells should be present if the film is sufficiently stained with brilliant cresyl blue A film that is poorly stained should be discarded Precipitated stain is often confused with reticulum, but may be recognized because it occurs outside as well as on the red cells it may be avoided by thorough washing after staining and frequent filtering of the cresyl blue and Wright s stain Platelets superimposed on red cells may also be confused with reticulum Reticulum is definitely blue whereas the granules of platelets are purple The amount of reticulum varies from a dense network of disconnected but multiple blue particles to one or two small particles Any cell containing material recognizable as reticulum is included in the reticulocyte count

The normal values for reticulocytes vary slightly with the method employed but are usually in the range of 0 5 to 1 5 percent The number of reticulocytes should be as great as or greater than, the number of polychromatophilic cells seen in a smear taken simultaneously but stained only with Wright s stain

4 *DETERMINATION OF VARIATION FROM THE NORMAL IN SIZE SHAPE AND HEMOGLOBIN CONTENT OF RED CELLS* A careful study of the blood film will show the variation from normal of the morphologic characteristics of the red cells in respect to size shape and hemoglobin content When these abnormalities are striking there is little difficulty in classifying the cells as macrocytic, normocytic or microcytic, and as normochromic or hypochromic When the changes are slight it is necessary to compare the cells with those of a normal individual

(*a*) Comparison with a Film of Normal Blood It is always possible to have at hand a normal film that may be interchanged with the abnormal film and observed under the microscope for comparison with an unknown film For a direct comparison with a normal film a satisfactory preparation of the unknown blood stained with Wright s stain is mounted on a glass slide with the blood film up A film of normal blood on a coverglass also stained with Wright s stain is then mounted with immersion oil stained side down on top of the unknown film The two films are then face to face and can be focused upon alternately and quickly, using the oil immersion objective so that the unknown and normal red cells can be compared for size, shape, and hemoglobin content The films are of necessity placed in this manner because in any other combination they are separated by too great a distance to permit focusing with the oil immersion lens on the lower film

(*b*) Determination of Mean Cell Volume

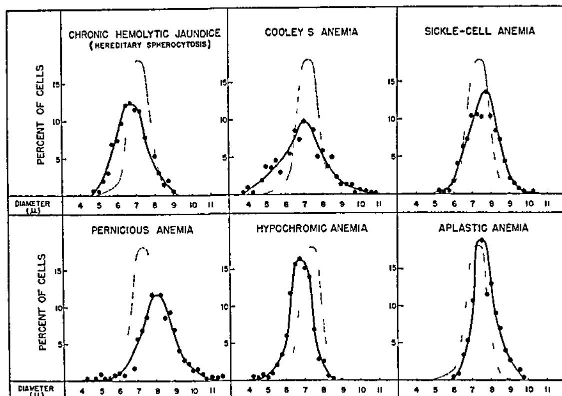


Fig 3 Typical curves of the f equency distribution of red-cell diameters in six examples of anemia compared in each inst nce to a curve (dotted line) derived from ten normal subjects (see Table 1)

TABLE 1 DATA FOR THE FREQUENCY DISTRIBUTION CURVES OF RED CELL DIAMETERS SHOWN IN FIG 3 *

Diag osis	Red ll ou t (10⁶/mm)	H m gl b gm/100 ml)	Hem toc t (per t)	M rp cul l m MCV (μ)	M corp scula h m gl b en io MCHC †	M ll d m t MCD (μ)	Sta d d d t on f d m	Coeff cient f n tio f d m t (pe t)	Mea ll th k ess MCT (μ)
	M l f 10 no mal dvid ls								
N rmal bj t	4 81	14 7	44 7	93	33	.2	0 50	7 0	2 3
	Ch ni hemolyti m s, h t zed by h editary d fects of th ed lls								
Cong tal (h) h m lyti j 3	3 34	10 0	29 7	89	34	6 83	0 80	11 8	2 43
Cooley emi	3 82	7 2	27 7	73	26	6 92	1 27	19 8	1 93
ickle-cell anemia	3 46	7 8	24 8	72	31	7 61	0 84	10 5	1 65
	A mias d t decreased prod tion f ed lls								
P rnicious mia	1 16	4 2	14 5	125	29	8 10	1 01	12 5	2 43
Hypoch m emi	2 72	4 2	16 7	61	25	6 70	0 64	9 5	1 74
Apl ti emia	1 48	6 0	16 1	109	37	7 7	0 71	9 2	2.34

Th d ces (m an corpuscul l m mea orpuscula h m gl bi tr ti d m corpuscul h m gl b) w cal ulated co ding to Wintrobe [305] Th mea ll diam er was measured cordi g t th m thod f Price-J es [234] ta da d dev and coeffici t f ariatio lso being determ ed. Th mea th ck ess f th ells was calculated fr m this mea l m d mea diam ter co ding to th m thod f on B ros [27] th ssumptio hat th ed ll is right ci cula cylind f k w lum and diam ter

† Grams f h mogl b p 100 ml f packed ed lls.

AND HEMOGLOBIN BY THE WINTROBE METHOD [305] Any blood that shows significant variation from the normal should have a red cell count hemoglobin and hematocrit with determination of the red cell indices of Wintrobe (MCV MCHC, MCH) These indices give the mean values for volume and hemoglobin concentration, but do *not give any estimate of the degree of variation* which is evaluated by visual examination of the film or by the Price Jones method discussed in the next paragraph These values should be compared and correlated with those observed in the films for size and hemoglobin concentration

(*c*) MEAN DIAMETER AND VARIATION OF DIAMETER AND THICKNESS OF RED CELL The mean size and variation in size of red cells can be determined from measurement of the cell diameters by the method of Price Jones [234] The data can be shown graphically as a distribution curve as in Fig 3 or analyzed statistically by determining the frequency of occurrence of diameters in each group (using differences of $\frac{1}{2}$ μ) The mean cell diameter (MCD) the standard deviation of the diameter from the mean and the coefficient of variation are determined The standard deviation of the diameters of a population of red cells expresses quantitatively the degree of variation from the mean diameter Since the absolute values for the mean diameter vary in different conditions the more useful statistical measure for comparison of results is the coefficient of variation from the mean diameter, which is expressed in percent (Table 1)

As shown in Fig 3 three hereditary anemias are characterized by abnormalities of the geometry of the red cells In congenital hemolytic jaundice the mean cell diameter is decreased so that the red cells appear microcytic on the blood film However, the mean cell volume (MCV) is normal or slightly increased so that the mean cell *thickness (MCT) is abnormally increased and* the cells are more spheroidal than normal, a fact that renders them more susceptible to osmotic and mechanical hemolysis [27, 40 113] In sickle cell anemia the mean diameter is increased so that the red cells appear macrocytic on the blood film. However, the mean cell volume is not increased but normal or slightly decreased so the thickness is decreased The increased flatness of these cells renders them less susceptible to osmotic hemolysis In Cooley s anemia, which is usually microcytic the coefficient of variation of the diameters is greater than in any other anemia and the microcytes predominate over the macrocytes

In pernicious anemia the coefficient of variation is also large greater than in any other macrocytic anemia [61] In many instances of aplastic anemia the cells may be macrocytic but the distribution of the diameters is approximately normal In hypochromic anemia the variation in diameter of the red cells is not so marked as in Cooley s anemia although the cells show an increasing degree of microcytosis and variation in diameter as the iron deficiency increases

6 PLATE II THE WHITE CELLS

1 *INTRODUCTION* The white blood cells are divided into five separate series namely granulocytic, lymphocytic, plasmacytic monocytic and histiocytic [53] The maturation and nuclear structure of the cells in each of the first four of these series are illustrated diagrammatically in Figs 4 5, 6 and 7 The structure of histiocytic cells is shown in Fig 8 It is not always essential when a differential count is made, to subdivide each series into as many stages as are shown in the figures but it is necessary to recognize these stages in order to understand the degree of maturity of each cell. In general the more immature the cells the more serious is the prognosis in diseases of the blood and in physiologic responses. Typical cells representing each stage are described It is not always possible to classify the cells since there are many intermediate stages and abortive and abnormal forms Sometimes the nucleus develops faster than the cytoplasm and sometimes the cytoplasm is more mature than would be expected with the structure of the nu

cleus Typical cells seen in normal blood are shown in Plate II

2 *GRANULOCYTIC SERIES* (Fig 4 and Plates II X and XI)

(*a*) NEUTROPHIL (polymorphonuclear neutrophil granulocyte Plate II *3 3a*) The neutrophil has a diameter of approximately 10 to 15 μ The nucleus shows two or more distinct lobes connected by bands or threads of chromatin and is made up of coarse condensed masses of purple staining chromatin The cytoplasm is pink to lilac The granules if present are fine and have the color of the nucleus (neutrophilic)

Abnormal forms Hypersegmented or multilobed neutrophils are found in untreated pernicious anemia although they may occur in other conditions Round or oval basophilic areas in the cytoplasm known as Dohle bodies may be seen in certain infections In certain other infections toxic granules which appear as blue black coarse granules may occur in the cytoplasm Occasionally these granules are mistaken for those of the basophil

(*b*) EOSINOPHIL The eosinophils are a little larger than neutrophils This is especially true when the cells are poured forth in large numbers as in certain infestations such as trichiniasis or other pathologic states The nucleus is usually bilobed or a band form (Plate II *5a 5b*) The cytoplasm is usually lilac to blue but may be difficult to see because of the large number of granules The granules are much larger than those seen in the neutrophil or in any other cell They are a bright orange red in color and sharply defined or round in outline They often appear over the nucleus as well as free in the cytoplasm

(*c*) BASOPHIL Basophils (Plate II *6a 6b*) are usually a little smaller than the mature neutrophil The nucleus is lobulated as in the neutrophil The cytoplasm is usually lilac to blue Large distinct blue granules are generally scattered throughout the cell and may be so numerous as to obscure the structure of the nucleus

(*d*) YOUNG GRANULOCYTE OR BAND FORM (stab young form Plate II *4*) The band forms of the neutrophil are immature cells which are often slightly larger than the mature forms with the corresponding granulation There may also be band forms of the eosinophil and basophil The nucleus of the cell is not segmented The cytoplasm is the color of the corresponding adult cell The granules are the same as those of the adult cell of the series being fine purple granules in the young neutrophil large blue granules in the young basophil (Plate II *6a*) and red orange granules in the eosinophilic cell The band form is the predominating cell in Pelger s anomaly [133]

(*e*) METAMYELOCYTE The nucleus is round indented or kidney shaped and the clumps of chromatin are not so deeply stained as in the older cells The cytoplasm and granules are similar to those of the corresponding adult cell

(*f*) MYELOCYTE C (late myelocyte) The nucleus is round and quite distinct in outline The smooth structure of the nucleus is contrasted with the clumped chromatin of the more mature forms The cytoplasm is more basophilic than in the metamyelocytes or later forms Granules may be neutrophilic eosinophilic or basophilic and if present are in the cytoplasm and are ***not*** seen over the nucleus

(*g*) MYELOCYTE B This cell is usually much larger than the more mature cells of this series (12 to 18 μ) The nucleus which may be round or oval is less sharply defined than in any other cell The chromatin structure is rather homogeneous The cytoplasm is more basophilic than in the older cells The granules are scattered throughout the entire cytoplasm and *cover* the nucleus Often the contour of the nucleus is obscured by the granules The granules may be neutrophilic eosinophilic or basophilic (or specific) The eosinophilic and basophilic granules are larger than the neutrophilic granules but smaller than the corresponding type of granule in the older cells (This is the earliest stage in which specific granulation appears)

(*h*) MYELOCYTE A (promyelocyte) The nucleus is a deep red purple with a fine granular structure Nucleoli may or may not be present A nucleolus is a small round sharply defined area in the nucleus that takes a light blue stain The cytoplasm is a deep blue A few scattered red purple or azurophilic granules appear These granules are usually nonspecific since differentiation into neutrophilic eosinophilic, and basophilic

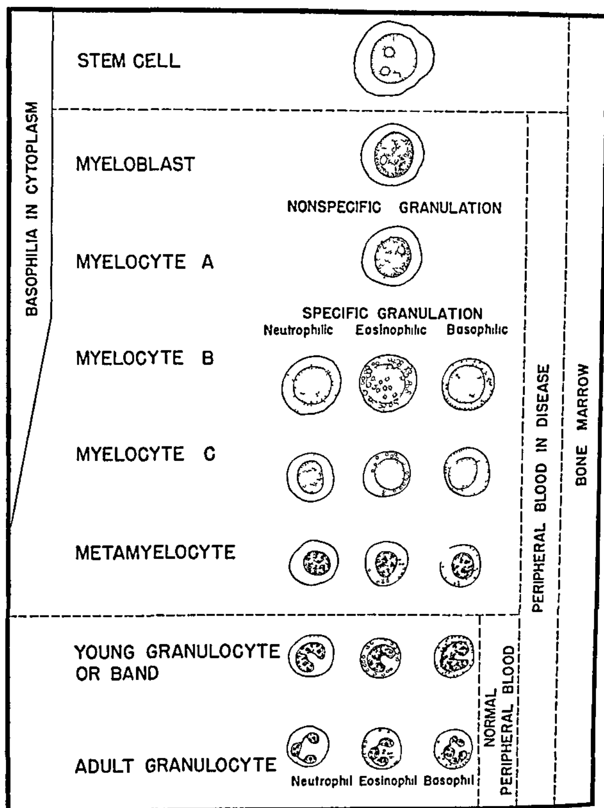


FIG 4 Diagram of the maturation of the granulocytic series

granules takes place at a more mature stage (myelocyte B)

(i) Myeloblast The nucleus is red purple with a finely granular or homogeneous chromatin structure There are usually one to three nucleoli present in the form of round blue areas The intensity of color of the nucleus is often less than that of the cytoplasm The cytoplasm is intensely basophilic No granules are present It may be impossible to distinguish the myeloblast from other blast forms

(j) Stem Cell (same as described above p 10)

3 *LYMPHOCYTIC SERIES* (Fig 5 and Plates II XIV and XVI)

(a) Small Lymphocyte (Plate II 7) The small adult lymphocyte is the smallest white cell (7 to 10 μ) The nucleus is deep purple and usually about the size of the red cell The round nucleus is made up of masses of chromatin so that no distinctive internal structure is seen The periphery of the nucleus is usually defined rather sharply The cytoplasm forms a narrow rim around the nucleus This cytoplasm should be a clear light blue but often in thick or heavily stained smears the color is rather dark Occasionally red purple granules and more rarely blue granules are present in the cytoplasm These granules however are few distinct and scattered and should not be confused with the finer granules of the monocyte

(b) Large Lymphocyte (Plate II 8) The large lymphocyte varies in diameter from 10 to 18 μ and may be as large as a monocyte The nucleus is usually somewhat larger than in the small lymphocyte and is often irregular in shape the chromatin masses are not clumped quite as much as in the small lymphocytes and may sometimes have a smooth appearance The cytoplasm is a clear light blue and is much greater in proportion to the nucleus than in the small lymphocyte Occasionally red purple granules may be scattered through the cytoplasm

(c) Young Lymphocyte The young lymphocyte occurs occasionally in normal bloods and frequently in children s blood and in the blood in infectious states This cell is the size of the large lymphocyte but the nucleus fills a greater proportion of the cell Chromatin masses of the nucleus are not so condensed as in the older forms but do not resemble the fine structure of a blast form A deep-blue basophilic cytoplasm is characteristic of these young cells No granules are present

(d) Lymphoblast The red purple nucleus is made up of finely granular chromatin with or without nucleoli as in other blasts The cytoplasm of the cell is deeply basophilic as is characteristic of other blast forms Sometimes there is a clear perinuclear area in the cytoplasm Occasionally blasts appear with almost no cytoplasm but containing the characteristic nucleus Granules are absent The lymphoblast may be indistinguishable from other blast forms

(e) Stem Cell (same as described above p 10)

(f) Atypical Lymphocytes (Plate XVI) Among the abnormal cells of the lymphocytic series those seen in infectious mononucleosis are of especial interest These cells vary markedly in their characteristics but three definite types may be recognized These abnormal forms may be seen in normal blood in small numbers but are seen in increased numbers in infectious mononucleosis infectious hepatitis chicken pox and serum sickness

One variety of atypical lymphocyte may be seen that is larger than the large lymphocyte and usually of irregular shape (appearing to have many sides) The irregular periphery gives the appearance of a distorted cell and the presence of so many of these irregularities of outline suggests that these cells are abnormally fragile The cytoplasm is a hyaline blue without granules The intensity of blue may be increased along the margin of the cell as if the cytoplasm were thicker or condensed along the edge The irregular nucleus is often more reticular and more wavy than that of the normal lymphocyte and for this reason may be confused with the monocyte

A second variety of atypical lymphocyte includes cells of large size and irregular shape similar to the first variety The chief characteristic of the second variety is the deep muddy blue color of the cytoplasm Although this basophilic cytoplasm is typical of immature cells or blast forms the nuclear structure is quite mature in appearance the nuclear chromatin occurring in wavy masses Granules are usually absent

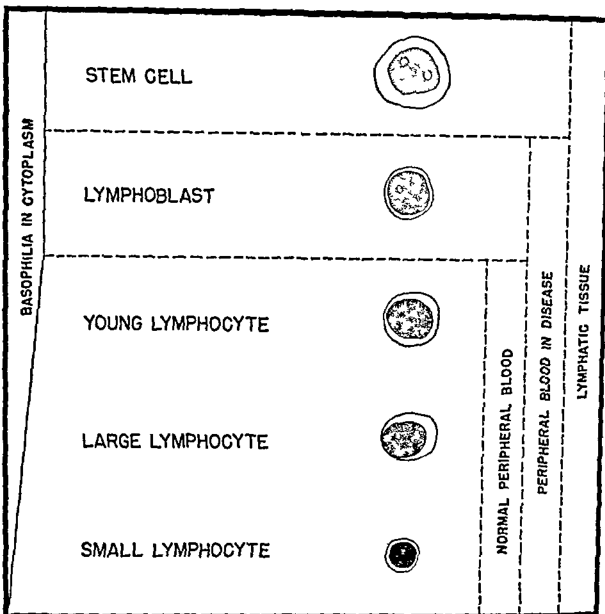


FIG 5 Diagram of the maturation of the lymphocytic series

In a third variety of atypical lymphocyte the cytoplasm is vacuolated in variable degree If the vacuolation is marked it may give a foamy appearance The cytoplasm may be light blue or a very deep blue

4 *PLASMACYTIC SERIES* (Fig 6 and Plate XV)

(a) PLASMA CELL (plasmacyte) The nucleus of the plasma cell (plasmacyte) has a very characteristic coarse deeply stained clumped chromatin structure This nucleus is usually eccentrically placed in an oval or elongated cell The cytoplasm is a deep green blue with a spongy appearance There are often vacuoles in the cytoplasm Globules or clear areas in the cytoplasm taking an acid stain are called Russell bodies These may occur singly or in groups, or they may rupture to make a larger irregular acidophilic

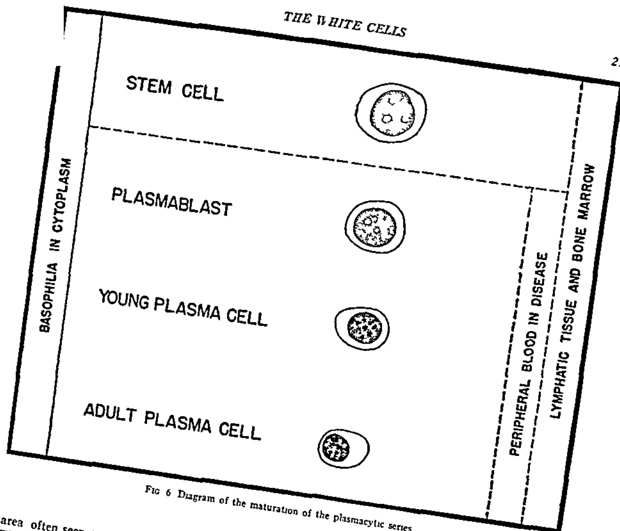


Fig 6 Diagram of the maturation of the plasmacytic series

area often seen around the edge of plasma cells The edge of the cytoplasm may be very ragged or fringed Granules are rare in the plasma cell but basophilic granules may occur Large basophilic granules which appear following treatment with stilbamidine are called inclusion bodies

(*b*) Young Plasma Cell The young plasma cell is usually larger than the adult plasma cell and has been described as the multiple myeloma cell The nucleus has a finer structure than that of the adult cell the chromatin often occurring in smaller clumps sometimes granular The nucleus is not necessarily eccentric in position The color of the cytoplasm is the same green blue as the adult cell but may be more intense The spongy appearance may be evident and vacuoles may be present

(*c*) Plasmablast The nucleus has a fine granular structure that is typical of all blasts One or more large sharply defined nucleoli are present The cytoplasm has the green blue color that is characteristic of this series of cells Vacuoles may be present The plasmablast may not be distinguishable from other blast forms in some instances

(*d*) Stem Cell (same as described above p 10)

(*e*) Abnormal Forms The presence of large irregular plasma cells with characteristic nuclei but with abundant cytoplasm and indefinite

ragged peripheries sometimes with streamers of cytoplasm, may be of significance and not artifactitious Although the eccentric nucleus is characteristic, it should be recognized that in pathologic conditions there are abortive forms and this is therefore, not an essential criterion Although the diameter of these cells varies from 10 to 20 μ the younger forms are larger than the adult cell In pathologic conditions such as plasmocytoma there are often abnormally large forms and sometimes cells with more than one nucleus

(*f*) COMPARISON OF PLASMA CELLS WITH OTHER CELLS The color of the cytoplasm in the plasmacytic series is a characteristic muddy green blue, the younger forms being a deeper, more intense blue This blue is much deeper than that seen in the cytoplasm of any lymphocyte and more of a green blue than that seen in other blast forms The cytoplasm of the plasma cytic series is sometimes confused with that of the monocyte but lacks the granules of the monocyte and is a much deeper blue and more opaque in appearance

5 *MONOCYTIC SERIES* (Fig 7 and Plate XII)

(*a*) MONOCYTE (Plate II *9*) The monocyte is larger than most of the other cells that are found in the normal peripheral blood (13 to 20 μ) The nucleus is indented or lobulated often presenting a folded appearance The structure of the nucleus is looser than that of any of the other cells as if it were made up of a network of chromatin Because of this lacy structure the nucleus appears to be stained lighter purple than the nuclei of other cells The cytoplasm is a gray blue, in contrast to the clear light blue of the lymphocytes The granulation varies in different cases The monocyte usually contains fine neutrophilic granules although in some cells red purple granules are scattered throughout the cytoplasm The amount of granulation varies from none to a dense stippling of fine granules

(*b*) YOUNG MONOCYTE (Plate II *10*) The nucleus in the young monocyte is round but the same lacy folded type of nucleus is seen as is present in the adult monocyte The cytoplasm is the same gray blue as in the adult form This cell should be compared with the adult cell in a given smear to differentiate the cell from the lymphocyte or the metamyelocyte The granules are the same as in the adult cell

(*c*) MONOBLAST The nucleus of the monoblast is much finer in structure than in the older cells of the series although the contour may be irregular and it may have a certain folded appearance characteristic of the adult cell Although the chromatin is finely granular, it is more lacy than in the other types of blasts Nucleoli are usually present The cytoplasm is a deep blue, showing more gray blue than in the myeloblast or lymphoblast There may be red purple granules in this blast form even in the presence of nucleoli Auer bodies, which appear as short red rods or splinters, are frequently present in the monoblast

(*d*) STEM CELL (same as described above p 10)

6 *HISTIOCYTIC SERIES* (Fig 8 and Plate XIII) The histiocyte, as seen in the peripheral blood and bone marrow represents a series of cells that are derived from the reticulo endothelial tissue throughout the body and can be differentiated from other series of white cells such as the monocytic

Histiocytes have been referred to by many terms such as large mononuclear leukocytes macrophages of Metchnikoff, *1892* endothelial leukocyte of Mallory 1898 adventitial cells of Marchand, 1898 clasmatocyte of Ranvier, 1900 and of Sabin Doan, and Cunningham 1923-24 histiocyte of Aschoff and Kiyono 1913 endothelial phagocyte of Foot, *1925* and monocyte by many investigators in recent years [*98 251 252*] Much experimental and clinical work on histiocytes is difficult to interpret because the cells were classified as monocytes

Histiocytes may be present in the peripheral blood film of normal individuals

The appearance of histiocytes varies greatly in the same blood film in different samples from the same individual and in samples from different individuals under pathologic conditions This variation in the type of cell seen may be related to the tissue from which the cells arise (spleen or lymph nodes) and to the process for which they are elaborated In histiocytic leukemia the histiocytes which may be markedly increased are probably the result of abnormal growth of a par

ticular cell type with proliferation in the peripheral blood as well as tissues No maturation cycle has been established for the histiocytic series Nucleoli may be present in cells that do not show basophilic cytoplasm while some of the most basophilic cells may lack nucleoli

Histiocytes are larger than the cells of the erythrocytic or white cell series and usually are markedly irregular in shape A few recognized types of histiocytes are described since no single description is adequate

(*a*) Histiocyte — Amoeboid Form The amoeboid form of histiocyte is larger than the monocyte or lymphocyte but is often confused with them (Plate XII *5*) The nucleus has a definite but delicate membrane and is lighter in color than that of the lymphocyte or monocyte The nucleus may appear as a loose network or as a structure resembling a honeycomb There may be one or more small blue nucleoli The abundant cytoplasm is a pale gray blue so delicate that the cell membrane is quite difficult to discern The irregular shape of the cell resembles an amoeba pseudopods of cytoplasm being very characteristic In some cells there appears to be endoplasm containing fine granules surrounded by a clear pale blue ectoplasm The granules if present are fine and red purple in color Irregular black particles or cellular debris may be present

(*b*) Histiocyte — Phagocytic Form The phagocytic histiocyte is very large (15 to 80 μ) and irregular in shape The nucleus is light purple and reticular sometimes with one or two nucleoli The cytoplasm is characterized by delicate lav

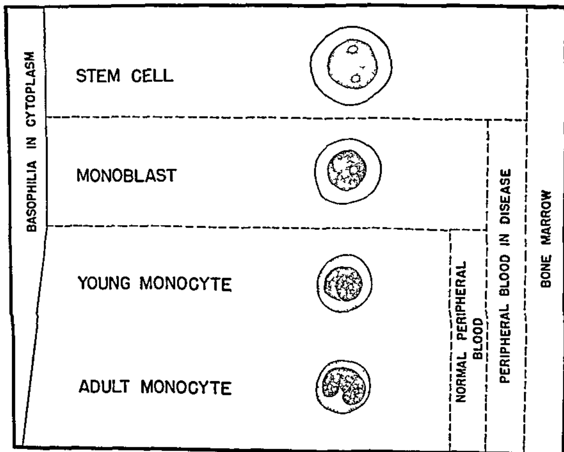


Fig 7 Diagram of the maturation of the monocytic series

ender veils In the cytoplasm there may be vacu oles as well as phagocytosed red cells, neutrophils platelets, or cellular debris

(c) Histiocyte — Basophilic or Leukemic Form (Plate XIII) The basophilic form of the histiocyte varies in size, but is larger than the other blasts The nucleus is often irregular in shape with uniformly granular structure, the granulation being less marked than in the other types of blasts Nucleoli may or may not be pres ent The cytoplasm in some cells is deep blue and very irregular in contour, sometimes it is much lighter than would be expected in a cell with a blast type of nucleus Red purple granules are of ten present even in these young forms

(e) Histiocytes in Lipoid Storage Diseases 8 and Plate XVI 4) Large irregular cells with deep blue cytoplasm often appear in association with an increase of lymphocytes and plasma cells and are usually classified as atypical lympho cytes It is difficult to identify the origin of these cells in some instances of virus infections and it is quite probable that they are histiocytes from lymphatic tissue rather than lymphocytes

(e) Histiocytes in Lipoid-Storage Diseases Large cells, irregular in shape and characterized by the spongy vacuolated cytoplasm sometimes seen in the lipoid diseases are histiocytes

(*f*) 'Hemohistioblasts of Ferrata Fer rata and Franco [93] described a large irregular cell frequently seen in the blood of patients with chronic myelogenous leukemia, as a hemohistio blast. The light blue cytoplasm may be clear, spongy, or granular The type of granules may be eosinophilic, neutrophilic, basophilic, or azurophilic. The nuclear structure is spongelike in character or like a honeycomb At least one nu cleolus is usually present These are also consid ered to be tis ue cells or histiocytes [245]

7 *DIFFERENTIAL COUNT OF WHITE CELLS* The differential count of white cells is one of the basic examinations of the patient It is used for diagnosis and in following the course of *the patient The white cell count and differential* white cell count in normal individuals, from vari ous sites are shown in Table 4 The quantitative limitations and significance of the results are dis cussed by Ham [116], Barnett [13], and others [106 182, 274] Normal variations in white cell counts under basic conditions are given by Stet son [277] A careful enumeration is made of the white cells on the film each type of white cell be ing classified In order that each cell may be counted only once the lide is moved progres sively and systematically from one field to the next as much of the film as possible being cov ered to obtain representative distribution The number of each class of white cells is determined and the results are expressed in percentage of the total number of white cells enumerated All abnormal white cells should be classified or de scribed in detail and reported Some of the differ ent types of abnormal white cells that may be seen in films of peripheral blood have been de

Fig 8 Diagram of histiocytes observed in peripheral blood in selected clinical cases

1 *Subacute bacterial endocarditis* These histiocytes show evidence of amoeboid activity and phagocytosis The cell in the center contains a red cell that has been phagocytosed A red cell is shown in the upper corner for comparative size

2 *Typhoid fever* Vacuoles are present in the nuclei

3 *Reticulum cell sarcoma* The histiocytes show vacuola tion and evidence of phagocytosis Nucleoli are present in the two lower cells A vacuole is pre ent in the nucleus and seven vacuoles occur in the cytoplasm of one of the upper cells There is vacuole in the cytoplasm of one of the other cell

4 *Severe anemia* The histiocytes in this blood film were very irregular in shape with pseudopods It can be seen in the upper cell how easily a portion of the cytoplasm can be separated and appear free in the peripheral blood These cells also show vacuolization

5 *Hodgkin s sarcoma* The predominating white cells in this blood film were large cells with deep-blue cytoplasm containing many vacuoles in both the nucleus and the cytoplasm

6 *Histiocytic leukemia* The histiocytes were varied in type in this blood many showing evidence of amoeboid ac tivity and phagocytosis

7 *Histiocytic leukemia* The histiocytes in this case showed evidence of marked amoeboid activity and phagocytosis of red cells (as seen in the cell at the left) and other foreign material (as seen in the two upper cells)

8 *Histiocytic leukemia* In this blood film the cells we much larger than a lymphocyte or lymphoblast The n clear structure was quite homogeneous but nucleoli we not present The cytoplasm was deep blue Evidence of a tive phagocytosis was seen

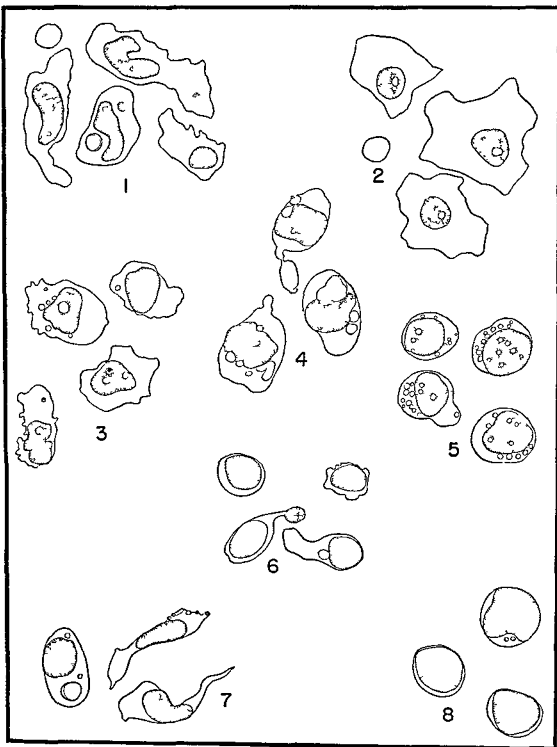


Fig 8 Diagram of histiocytes observed in peripheral blood in selected clinical cases

scribed in the preceding paragraphs and are summarized in Table 2 Some other differential points in identifying the early cells are given in the next section Cells that are ruptured, fragmented, or degenerated ("basket" cells or "smudges") usually cannot be identified and are not included in the differential count but may be noted separately and reported as the number seen per 100 white cells A significant number of broken cells may be seen in leukemic bloods The counts are performed in multiples of 100 cells using one or two coverglass preparations At least 100 cells should be counted as a screening procedure

When nucleated red cells are present in the blood film they should be reported as the number seen while counting 100 white cells but they are *not* included in the differential count of leukocytes If there are large numbers of nucleated red cells present, the stage in the erythrocytic maturation series should be noted an actual count made of the number of nucleated forms per 1000 red cells and the percentage determined From this figure the absolute number of nucleated red cells per cubic millimeter is calculated

8 *DISTINGUISHING CHARACTERISTICS OF IMMATURE CELLS FREQUENTLY CONFUSED* The recognition of the blast form and its differentiation from the more mature cells is of utmost diagnostic importance Blasts have in common the characteristics of deeply basophilic cytoplasm, nuclei having finely and homogeneously granular chromatin structure, and frequent presence of nucleoli In general the presence of blasts in the peripheral blood is a sign of marked abnormality of the blood forming organs and should lead to more extensive and repeated blood examinations Nucleated red cells occurring in the peripheral blood at approximately a normal level of red cells may indicate extramedullary myelopoiesis or myelophthisis due to leukemia lymphoma or neoplastic invasion of the bone marrow

The blasts of red cells should be readily distinguished from the blasts of white cells which often occur in the same blood film since proerythroblasts or early erythroblasts with basophilic cytoplasm are usually present in association with the more mature nucleated red cells with polychromatophilic or hemoglobin containing cytoplasm *In* the proerythroblast the cytoplasm is more of a deep gray blue than that seen in the white cells the nucleus is usually definitely round and more

TABLE 2 CHARACTERISTICS OF CELLS THAT ARE FREQUENTLY CONFUSED

Cells to be distinguished	Diameter (μ)	Cytoplasm		Nucleus				Granules	
		Color	Amount	Shape	Structure	Color	Nucleolus	Color	Number
Monocyte	13–20	Gray blue	Abundant	*Adult* Indented *Young* Round	Reticular lacy folded	Blue-purple	0	Purple and red purple	Few to many peppered
Lymphocyte									
small	7–10	Clear light blue	Scant	Round	Condensed chromatin	Deep purple	0	Red-purple rarely blue	Few scattered
large	10–20		Abundant	Round		Deep purple	0		
Neutrophil									
adult	10–15	Pink to lilac	Moderate	Segmented	Condensed chromatin	Purple	0	Fine purple	Few to many
young (band)	10–18		Moderate	Nonsegmented		Purple	0	Fine purple	*Few to many*
Metamyelocyte	12–20	Pink to lilac	Moderate	Round or indented	Slightly clumped	Purple	0	Fine purple	Few to many
Myelocyte C	15–20	Gray blue	Moderate	Round	Homogeneous	Purple	0	Purple	*Few to many*
Histiocyte	15–50	Light gray blue spongy	Abundant irregular	Irregular or round	Honeycomb vacuolated	Purple	±	Varied, also phagocytic particles	Rare
Plasma cell	10–25	Gray green blue	Abundant	Round	Wedges of chromatin	Deep purple	0	0	Rare
Proerythroblast	15–25	Deep blue	Small	Round	Homogeneously granular	Red-purple	+	0	0
Early erythroblast	10–20	Gray blue	Moderate	Round	Finely divided chromatin	Purple	0	0	0
Late erythroblast	10–20	Blue to lavender	Moderate	Round	Coarse lumping of chromatin	Purple	0	0	0
Normoblast	7–15	Pink to lavender	Variable	Round	Condensed chromatin	Deep purple	0	0	(stippling)

red purple than in the other blasts nucleoli may or may not be present

The recognition of the type of blast of the white cell series is usually of only academic interest Identification may be difficult or impossible and usually contributes little to the welfare of the patient If blasts are present in association with more mature forms this is presumptive but not definite evidence that the immature cells belong to this same series Immature cells of the lymphocytic and plasmacytic series may be present in the same blood film in such a disease as infectious mononucleosis In stem cell leukemia or in some acute leukemias becau e of the abortive cells present differentiation is not possible With a leukopenia the number of blast cells may be so small that the identification of the series to which they belong may be impossible When a buffy coat or a concentration of these cells is made before preparing a film the cells may be distorted so that the series to which the blast belongs can not be determined The myeloblast and lymphoblast are quite similar although the lymphoblast often has less abundant cytoplasm The plasma blast has the green blue basophilic cytoplasm that is characteristic of the mature forms of the series The monoblast is usually larger than either the lymphoblast or the myeloblast the nuclear structure is more reticular than in the other types of blasts the nucleus is often folded or irregular in shape granules are sometimes present in the deep gray blue cytoplasm The size of the cells the basophilia of the cytoplasm the structure of the nucleus and the presence of nucleoli should differentiate the blast forms from lymphocytes which when mature have a nucleus about the size of a red cell For the distinguishing characteristics of other cells that are easily confused see Table 2

7 THE PLATELETS AND THE THROMBOCYTIC SERIES

1 *ORIGIN* Although there have been many theories about the origin of platelets that presented by Wright [311] has been the most generally accepted Wright showed that small hyaline bodies containing red purple granules were detached fragments or pseudopods of the megakaryocytes of the bone marrow that appeared as free formed elements in the peripheral blood The differential staining property of Wright s modification [312] of the Romanowsky stain was an important factor in establishing the relation between the granule containing cytoplasm of the megakaryocyte and the platelets Other evidence given in support of Wright s theory of platelet formation is (*a*) platelets are not seen in the embryo until after the appearance of the megakaryocytes (*b*) blood platelets and megakaryocytes are found only in mammals (*c*) the number of platelets in the blood roughly parallels the number of megakaryocytes in the bone marrow in certain di eases of physiologic conditions Wislocki Bunting and Dempsey [310] have shown with histochemical methods that there are points of similarity between blood platelets and the cytoplasm of megakaryocytes The evidence indicates that platelets are cytoplasmic rather than nuclear in origin and probably arise from megakaryocytes

2 *DESCRIPTION OF NORMAL AND ABNORMAL FORMS OF THE THROMBOCYTIC SERIES* (Fig 9)

(*a*) PLATELET OR THROMBOCYTE The platelets are formed elements of the blood measuring 2 to 5 μ in diameter that appear as a clump of red purple (azurophilic) granules in a light blue cytoplasm that is almost transparent Although true nuclei do not occur in platelets the granules may be aggregated in a sharply outlined mass in the central part of the platelet re embling a nucleus When the blood film is vitally stained with brilliant cresyl blue before the Wright s stain as for reticulocytes the cytoplasm is intensely stained with the blue Sometimes the platelet is devoid of granules appearing as an irregular mass of cytoplasm At other times the platelet contains large numbers of granules The cytoplasm may be intensely blue in some instances probably a sign of immaturity These apparently young forms

BASOPHILIA IN CYTOPLASM

STEM CELL



MEGAKARYOBLAST



YOUNG MEGAKARYOCYTE



ADULT MEGAKARYOCYTE



BONE MARROW

MEGAKARYOCYTE



PERIPHERAL BLOOD IN DISEASE

PLATELETS



NORMAL PERIPHERAL BLOOD

Fig 9 Diagram of the maturation of the thrombocytic series Platelets are shown diagrammatically at the periphery of the megakaryocyte nucleus (Only platelets are seen normally in the peripheral blood)

are associated with states of thrombocytopenia or with the sudden increase in number of platelets following hemorrhage or splenectomy [180]

The size of the platelet varies from the normal to abnormally large forms almost the size of a red cell In normal individuals approximately 82 per cent of the platelets are 2 5 μ in diameter or less [217] In cases of thrombocytosis the deviation from normal is usually due to an increase of small platelets Increase of large platelets is often associated with intense regenerative activity abnormal function or hyperplasia of the megakaryocytes in the bone marrow Although the total number of platelets is abnormally decreased large platelets are characteristic of the blood picture in aplastic anemia untreated pernicious anemia paroxysmal nocturnal hemoglobinuria chronic lymphocytic leukemia acute leukemias or the acute exacerbation of chronic leukemia [167 291] Giant platelets are seen in increased numbers in polycythemia vera [194] and in chronic nonleukemic myelosis [37] Some most unusual forms of platelets have been seen in a few patients with myeloid metaplasia in whom splenectomy had been performed [57]

A decrease in the number of platelets is seen as a result of large doses of benzol following certain drugs and toxins [83] and following radiation [180] In infections there may be a decrease of platelets followed by a rapid increase [240 291] It is well known that platelets increase rapidly following hemorrhage [217] splenectomy [161 168] or other surgical operations childbirth and fractures [71 217] The number of platelets has been increased to more than $1000 \times 10^3/mm^3$ (normal value 200 to $400 \times 10^3/mm^3$) following splenectomy for purpura hemorrhagica [105] and after other surgical procedures Occasionally an increase in platelets has been associated with postoperative thrombosis and pulmonary embolism [71 167] In myelogenous leukemia the platelets are frequently normal or increased In 7 of 35 cases in one study [195] the platelets were abnormally increased (800 to $2000 \times 10^3/mm^3$) When the platelets are increased there may also be many young and abnormal forms of platelets with fragments of megakaryocytes appearing in the peripheral blood Marked increase or marked decrease from the usual level of platelets in a patient with myelogenous leukemia is a sign of change in the course of the disease

(*b*) Megakaryocyte (peripheral blood) The megakaryocyte may be seen in the peripheral blood in certain pathologic conditions such as myelogenous leukemia, agnogenic myeloid metaplasia polycythemia pneumonia and sepsis [193] The nucleus is practically all that is seen of this large distorted cell when it reaches the blood stream The diameter of the nucleus may vary from that of a red cell to two or three times that of a monocyte The nucleus of the megakaryocyte has a blue purple wavy chromatin structure with a dark periphery Sometimes there is a folded or lobulated appearance to the nucleus The light blue cytoplasm is rarely seen intact but appears torn and fragmented usually containing a mass of blue purple or azurophilic granules Platelets that appear as fragments of this granule containing cytoplasm are often seen in masses attached to or in the vicinity of the megakaryocyte

(*c*) Megakaryocyte (bone marrow) As indicated by its name the megakaryocyte is the largest cell of the bone marrow series (2 to 40 μ) but varies widely in size and appearance The nucleus is lobulated and is much more dense and deeper blue purple than the younger cells of the megakaryocyte series The cytoplasm varies in color from blue to pink and contains a variable number of characteristic red purple granules Fragments of cytoplasm are seen breaking off from the cell Some of these fragments of cytoplasm contain granules and look like platelet material Platelets are usually associated with these cells

(*d*) Promegakaryocyte (bone marrow) The promegakaryocyte is smaller than the megakaryocyte The nucleus is round or beginning to be lobulated The cytoplasm is more basophilic than in the older form Granules are present but are usually fine and scattered around the nucleus

(*e*) Megakaryoblast (bone marrow) The megakaryoblast is larger than the other blast forms in the bone marrow but smaller than the adult megakaryocyte The nucleus is slightly irregular and the chromatin is granular but coarser in structure than in the myeloblast One or more

II Endocrine deficiency
   A Deficiency of thyroid, adrenal and pituitary hormones
III Mechanical interference of marrow function
   A ? Inadequate marrow capacity
      Anemia of newborn
      Anemia of prematurity
   B Myelophthisis (Plates XI, XII, XIII, XIV and XV)
      Leukemia
      Lymphoma
      Myelofibrosis
      Osteosclerosis
      Metastatic carcinoma
      Primary xanthomatosis
      Miliary tuberculosis
IV Relative inhibition of marrow
   Chronic infection
   Chronic renal disease
   Cancer
   Cirrhosis
   Collagen diseases
V Complete bone marrow failure (aplastic or hypoplastic)
   A Congenital
   B Acquired
      1 Due to chemicals toxic to normal marrow
         Mitotic poisons (mustards benzol etc)
         Antimetabolites
      2 Due to chemicals toxic to hypersensitive marrow
         Arsenobenzols
         Chloramphenicol
         Gold
      3 Due to physical injury
         Radiation
      4 Idiopathic

9 PLATE III PERNICIOUS ANEMIA

1 *DESCRIPTION OF UPPER PICTURE BEFORE TREATMENT (WRIGHT S STAIN)* This is definitely the picture of a macrocytic anemia because the average diameter of the red cells is greater than normal (compare with normal cells (*3*) and see also Fig 1 and Plates I and II) As inspection shows the variation in size is extreme from the tiny microcytes (*11*) to oval macrocytes (*9*) the variation in size may be measured according to the method of Price Jones [234] as shown in Table 1 and Fig 3 There is marked variation in shape with many irregular forms (*13*) the most characteristic type being the pear shaped or tailed forms (*12*) Most of the cells are well filled with hemoglobin, as judged by the depth of staining A few of the small cells are definitely hypochromic (pale staining) Polychromatophilic cells (*8*) and stippled cells (*7*) are present Note that the stippled cells are stained by using Wright s stain alone and are not to be confused with reticulocytes (see lower picture)

There are two white cells in the field — a typical small lymphocyte (*2*) and an abnormally large multilobed neutrophil (*1a*) The platelets (*14*) occur singly and are definitely decreased in number

2 *DESCRIPTION OF LOWER PICTURE SIX DAYS AFTER INITIAL TREATMENT WITH LIVER EXTRACT* The film is stained with brilliant cresyl blue to show the reticulocytes and counterstained with Wright s stain In this picture the red cells show the same degree of macrocytosis and variation in size and shape as in the upper picture before treatment The hemoglobin concentration of the red cells in the lower picture is more variable than in the upper picture, as is evidenced by the variable intensity of staining This is especially noticeable in the macrocytic reticulocytes which are sometimes less densely stained than many of the nonreticulated red cells The red cells containing a network of blue staining reticulum (*6a 6b 6c*) are the reticulocytes The reticulocytes are numerous, and their age (in the peripheral blood) can be estimated roughly by the amount of reticulum contained in the cells Thus a large amount of reticulum (*6a*) indicates the very young cell As the cell matures, the amount of reticulum decreases (*6b*) until there are only traces of it re

maining (*6c*) The normoblast (*4*) and erythroblast (*5*) are immature nucleated forms of the erythrocytic series that contain reticulum and should be counted both as reticulocytes and as nucleated red cells (see Plate I)

The white cells in the lower drawing include a normal lymphocyte (*2*) and a normal neutrophil (*1*) The platelets are plentiful

3 *INTERPRETATION OF BOTH PICTURES* The marked macrocytosis and extreme variation in size of the red cells are consistent with the diagnosis of pernicious anemia in relapse in which the variation in size is greater than in any other macrocytic anemia [61] Among the macrocytes can be seen many oval macrocytes (*9*) that are well filled with hemoglobin These are especially characteristic of pernicious anemia and are present in large numbers when the red cell count is very low

Well formed microcytes (*11*) full of hemoglobin and tailed or pear shaped forms (*12*) are also characteristic of this disease The number of polychromatophilic red cells (reticulocytes) and nucleated red cells in untreated cases is small but is increased within about 2 days and reaches the maximum within 7 to 10 days following effective therapy (lower picture) or in spontaneous remission which rarely occurs Stippled cells are common as they are in many severe anemias Cabot rings and Howell Jolly bodies in the red cells are found frequently (not illu trated in these pictures)

Before therapy the neutrophils are decreased in number and many of them are abnormally large cells with multilobed nuclei which are referred to as mutilobed neutrophils or macropolys Because of the decreased number of neutrophils lymphocytes appear to be increased although their absolute number is within normal limits this is known as relative lymphocytosis After effective therapy the neutrophils quickly return to normal in appearance and in number

Before therapy the platelets are single large and decreased in absolute number they return to normal following treatment

When the anemia is severe (red cell count 0 5 to $2\ 0 \times 10^6/mm^3$ these abnormal red-cell characteristics are striking and study of the blood film is of great diagnostic value However, when the anemia is less severe (red cell count 2 0 to $3\ 0 \times 10^6/mm^3$) these features are present to a lesser degree and the diagnosis is more difficult At higher levels of the red cell count (above 3 0 $\times 10^6/mm^3$) some of the abnormal features such as oval macrocytes microcytes tailed forms stippling large platelets and mutilobed neutrophils may be present but in decreased numbers

The macrocytosis and the degree of variation in size of red cells are more striking in Addisonian pernicious anemia than in the other macrocytic anemias that respond to liver extracts folic acid and vitamin B_{12} such as the macrocytic anemias of pregnancy and those of nutritional (extrinsic factor) deficiency [61]

In the hemolytic anemias there may be marked macrocytosis and considerable (but not extreme) variation in the size of the red cells in such conditions as severe and chronic acquired hemolytic jaundice acute exacerbations of chronic hemolytic jaundice and paroxysmal nocturnal hemoglobinuria In these conditions however without any treatment the blood film shows marked polychromatophilia (with Wright s stain) and reticulocytosis (with brilliant cresyl blue) as shown in Plate VII The macrocytes are round and many are polychromatophilic. Oval macrocytes are seldom seen in these hemolytic anemias The platelets and granulocytes are usually normal and not decreased as in pernicious anemia except in paroxysmal hemoglobinuria of the nocturnal type

In certain macrocytic anemias that are refractory to liver extract and result from decreased production of red cells there may occasionally be moderate to marked macrocytosis This may occur in myelophthisic anemia due to leukemia and in the anemias as ociated with azotemia and chronic liver disease In these conditions the variation in size of red cells is moderate and the number of reticulocytes is low compared to that of the hemolytic anemias

10 PLATE IV HYPOCHROMIC ANEMIA (Iron-deficiency Anemia)

1 *DESCRIPTION OF UPPER PICTURE MODERATE HYPOCHROMIC ANEMIA IN A 17-YEAR-OLD GIRL* This is the picture of a moderate microcytic anemia with many red cells that are slightly smaller in diameter than the normal cells (*4*) There is moderate variation in shape with a few elongated (pencil) forms (*7*) and a few irregular forms Microcytes (*6*) are present and one polychromatophilic cell (*9*) The hemoglobin concentration of the red cells is slightly below normal This may best be judged by comparing the intensity of staining with that of a group of normal red cells as seen in Plate I

The white cells are normal and include a small lymphocyte (*2*) and a neutrophil (*1*) The platelets (*13*) are normal in number

2 *DESCRIPTION OF LOWER PICTURE SEVERE HYPOCHROMIC ANEMIA FROM CHRONIC BLOOD LOSS (HEMORRHOIDS) IN A 62-YEAR-OLD MAN* Many of the red cells in this drawing are microcytic and hypochromic However the variation in size here is extreme from two large target cells (*8*) which are probably thin cells to small microcytes (*6*) Also the variation in shape is extreme as evidenced by the many irregularly shaped cells and some pencil forms Polychromatophilic cells (*9*) a normoblast (*11*) and a stippled cell (*10*) are present Most of the red cells are definitely hypochromic or low in hemoglobin concentration as indicated by the pallor of the cells The hemoglobin appears to be concentrated about the periphery of these thin cells so that the color seems to decrease gradually from the periphery toward the center These progressive changes are in contrast to the sharply defined punched-out areas of central pallor that appear to result from artifacts in fixation or staining of the film

Two normal white cells are shown in this picture a neutrophil (*1*) and a monocyte (*3*) The platelets are large and abnormal in shape as often occurs in a severe anemia especially of a chronic type They appear to be approximately normal in number relative to the red cells on the film but the platelets may be decreased in absolute number since the red cell count is low

3 *INTERPRETATION OF BOTH PICTURES* The contrast between these two blood films is so extreme that it suggests different disease processes However both represent different degrees of chronic hypochromic microcytic anemia due to iron deficiency In chronic hypochromic anemia the greater the anemia or the lower the hemoglobin concentrations the more abnormal is the appearance of the red cells

The upper picture is typical of the changes occurring in the many instances of mild hypochromic anemia Since the morphologic changes are subtle the condition may be overlooked The direct comparison of the patient s blood film with that of a normal person will aid materially in detection of moderate changes (*Atlas* Section 5 4a) Also unless the red cells are well separated on the blood film and well stained the moderate changes may be missed However, the lower picture represents extreme changes in the red cells resulting from chronic iron deficiency due to blood loss over many years

Usually there is little difficulty in making a correct diagnosis of hypochromic anemia in such cases as those shown here if the appearance of the blood film is correlated with the red cell indices and the reticulocyte count Thus in both of these cases the values for mean corpuscular volume MCV indicated microcytosis and those for mean corpuscular hemoglobin concentration MCHC indicated hypochromia The variation in the diameters is extreme in the lower picture In both of these cases the reticulocytes are significantly few indicating decreased production of red cells by the bone marrow The level of reticulocytes is lower than would occur in hemolytic anemias including Cooley s anemia

4 *OCCURRENCE* Hypochromic red cells are seen in the film wherever iron deficiency follows blood loss as in neoplasms ulcers and parasitic infestations of the gastro-intestinal tract and in menorrhagia etc. In the more severe and chronic conditions the red cells become microcytic as well as hypochromic Hypochromic anemia may occur when the iron requirements for hemoglobin formation exceed the iron intake during periods of

rapid growth as in the first year of life during adolescence and during pregnancy Although anemias associated with infections are usually normocytic and normochromic slightly hypochromic microcytic anemias may occasionally be found in chronic active infections such as tuberculosis rheumatic fever osteomyelitis and chronic arthritis [306]

Hypochromic microcytic red cells are seen in the blood films of patients with polycythemia vera These changes in the red cells may be due to previous hemorrhages from ulcers or to iron deficiency produced by repeated therapeutic venesections

11 PLATE V COOLEY'S ANEMIA (Thalassemia Major, Mediterranean Anemia)

1 *DESCRIPTION* In this picture the red cells show extreme variation in size shape and hemoglobin content A few red cells appear to be normal in size (*4*) but the majority are microcytes The single most striking feature of the erythrocytes is their extreme variation in shape which exceeds that usually found in severe anemias and includes many biazarre shapes Because of the marked variation in the hemoglobin content of cells there is a marked variation in the intensity of staining of these cells A few cells appear well filled with hemoglobin but most of them have a decreased amount of hemoglobin which appears to be distributed around the edge of the cell as evidenced by the concentration of stained material such as is seen in hypochromic anemia (Plate IV) Many target forms (*8*) [293] are present Polychromatophilic cells (*9*) and nucleated red cells are increased A normoblast with a multilobed nucleus (*13*) a late erythroblast (*14*) and an early erythroblast (*15*) are shown Cells with Cabot rings (*11*) stippling (*10*) and Howell Jolly bodies (*12*) are present

Immature leukocytes include a myelocyte (*3*) and a young lymphocyte (*2*) The platelets (*17*) appear to be normal

2 *INTERPRETATION*

(*a*) Cooley s Anemia This blood film is from a 5 year old child of Italian parentage who showed increased fatigability pallor recurrent attacks of abdominal pain mongoloid facies enlargement of the liver and spleen and acholuric jaundice

The changes in the red cells in this picture do not differ greatly (except for the presence of polychromatophilic cells — elevated reticulocytes) from those of severe hypochromic anemia as seen in the lower picture of Plate IV From the blood films alone these two conditions may not be distinguishable However it is important to recognize from the data given in Table 5 that the marked changes in red cells occur at a high level of the red cell count $3\ 3 \times 10\ /mm^3$ and at a hemoglobin level of 5 8 gm/100 ml Conversely in the severe hypochromic anemia with comparable red cell changes the red cell count is markedly low $1\ 2 \times 10\ /mm$ and the hemoglobin level is excessively low 2 0 gm/100 ml In Cooley s anemia the absolute number of reticulocytes is consistently elevated above normal while in severe hypochromic anemia untreated the absolute number of reticulocytes is decreased below normal [29 300]

This blood film might be confused with that of pernicious anemia because of the marked variation in shape of cells However it should be re emphasized that the red cells in Cooley s anemia are hypochromic and microcytic whereas those of pernicious anemia are normochromic and macrocytic

(*b*) Cooley s Trait The blood picture in Cooley s trait is similar to that of a mild hypochromic anemia showing microcytosis hypochromia and moderate variation in size and shape In addition there are pencil forms target forms frequent stippled cells rare nucleated red cells and fewer than 4 percent of reticulocytes The red cell count varies from $5\ 0$ to $8\ 0 \times 10^6/mm$ and the mean corpuscular volume is definitely below normal The values for mean corpuscular hemoglobin concentration are higher for a given cell size in Cooley s trait than in hypochromic anemia A comparison of the blood picture of Cooley s anemia with that of Cooley s trait may well be made

by studying Plate IV (hypochromic anemia of iron deficiency) assuming that the red cell counts are about $5 \times 10^6/mm^3$ in both instances The final diagnosis depends on the lack of response to iron therapy and a careful study of the blood in other members of the family Clinically the subjects with Cooley's trait are usually in normal health and it is only by careful laboratory studies that the presence of the trait is found A comparison of the clinical and hematologic findings is given in the report by Daland and Strauss [62]

(c) COOLEY'S ANEMIA AND COOLEY'S TRAIT The genetic relation and the clinical differentiation between Cooley's anemia (thalassemia major) and Cooley's trait (thalassemia minor) have been discussed by many investigators [36 62 65 66, 186 216 292, 307] It is probable that Cooley's anemia is the result of the Cooley factor's being present in the homozygous state inherited from both parents The disease is characterized by a severe anemia and is usually considered to be a fatal disease during childhood but a few cases have been recognized in patients who have survived to the third and fourth decade [62, 183]

Cooley's trait appears to be due to the occurrence of the Cooley factor or gene as a heterozygote Clinically the carriers of the trait are asymptomatic although the blood values are abnormal

Individuals with unusual forms of anemia fulfilling some of the criteria for Cooley's anemia have been observed These abnormalities are the result of combination of the Cooley factor and an abnormal hemoglobin Electrophoresis and various chemical methods now permit the identification of certain abnormal hemoglobins which have been designated by letters in the order of their recognition [243, 271 276] Individuals have been found who are simultaneously heterozygous for Cooley's trait and for an abnormal hemoglobin such as Cooley's factor with hemoglobin S [16 45 134, 165 214 233, 261, 266 269] hemoglobin C [266, 267 315] hemoglobin E [43 174, 282] and hemoglobin G [258] In general when two different abnormalities are heterozygous the clinical and hematologic manifestations are more severe than when either defect is present alone but not as severe as when either of the abnormal factors is homozygous The hematologic observations of such cases are summarized in Table 3

Fetal hemoglobin (hemoglobin F), a normal major constituent of embryonic blood decreases to less than 5 percent during the first few years of life [140] In certain pathologic states this fetal hemoglobin remains abnormally high In Cooley's anemia 50 to 90 percent of the hemoglobin may be of the fetal type [41 44] as determined by the method of alkali denaturation [264]

The original studies of Minnich *et al* [192] in Thailand and of Lie Injo [174] in Indonesia showed a high incidence of apparent Cooley's anemia Further studies with improved methods revealed the presence of hemoglobin E in some of these families [42 43 173 208] Some of these individuals were heterozygous for hemoglobin F some homozygous for hemoglobin E and in some the Cooley factor and hemoglobin E were both heterozygous Homozygous E produces an anemia similar to Cooley's anemia

Other abnormal hemoglobins such as H and A are found in anemias that are similar to Cooley's anemia and Cooley's trait [4 163 204, 246] Further investigation in this field is in progress

12 PLATE VI SICKLE-CELL ANEMIA

1 *DESCRIPTION OF UPPER PICTURE* (unmanipulated film of peripheral blood from a 13 year old Negro girl) The red cells in this film of peripheral blood show more than normal variation in size Many of the red cells appear to be a little larger than normal [73] and show more than normal variation in shape with microcytes polychromatophilic macrocytes (5) and target cells (6) The number of crescent or elliptic forms (7) and oat shaped forms is striking these cells probably represent irreversible sickled forms [259] that have lost their filamentous processes There is marked variation in hemoglobin concentration as manifested by the variation in intensity of

TABLE 3 A SUMMARY OF MORPHOLOGIC HEMATOLOGY IN THE HEMOGLOBINOPATHIES

Condition	Type of hemoglobin	Anemia	Hemoglobin concentration	Size	Reticulocytes	Nucleated red cells	Target forms	Howell-Jolly bodies	Stippling	Sickled form Fixed prep	Sickled form Wet prep
Sickle-cell trait	A S	0	Norm	Norm	N	0	+	0	0	0	+++
Sickle-cell anemia	S S F	+++	Norm	Norm Micro	++	++	++	++	++	+ to +++	+++
Sickle-cell and Cooley's	S A F	+++	Hypo	Micro	++	+++	++	+	++	±	+++
Sickle-cell and Hemoglobin C	S C F	+ to +++	Norm	Micro	+	+	+++	+		±	+++
Sickle-cell and Hemoglobin D	S D F	+++	Norm	Micro	++	+	±			+	+++
Sickle-cell and Hemoglobin G	S G	++	Norm	Norm	+		±			+	+++
Hemoglobin C — heterozygous	A C	0	Norm	Norm	N	0	+ to +++			0	0
Hemoglobin C — homozygous	C C F	++	Norm	Norm or Micr	+	±	+++			0	0
Hemoglobin C — Cooley	C A	+++	Hypo	M	+		++			0	0
Hemoglobin D — heterozygous	A D	0	Norm	Norm	N	0	0			0	0
Hemoglobin E — heterozygous	A E	0	Norm	Norm	N	0	0			0	0
Hemoglobin E — homozygous	E E F	+	Norm	Micr	+	±	++			0	0
Hemoglobin E — Cooley	E A F	++++	Hypo	Micro	++	+++	++			0	0

Hemoglobins included A (Adult) F (Fetal) S (Sickle) C, D and E
N represents [illegible] greater than 2 per cent.
Norm = normochromic Hypo = hypochromic
N rm = normocytic Micro = microcytic, M cro = macrocytic
Fixed preparations stained with Wright's stain w [illegible] prepared [illegible] with sodium metabisulfite

staining The presence of many target cells which are large thin cells gives the appearance of a lowered hemoglobin content although the mean corpuscular hemoglobin concentration (MCHC) is only slightly reduced The nucleated red cells, which in this film are normoblasts (*8*) and late erythroblasts (*9*) appear in a crisis in sickle cell disease or may be present in the blood in patients showing chronic anemia of moderate severity

Only one white cell a normal lymphocyte (*6*) is shown Platelets appear to be normal

2 *DESCRIPTION OF LOWER PICTURE* (film of reduced anoxic venous blood from a 22 year old Negro male) Venous blood was reduced by equilibration with 90 percent of nitrogen and 10 percent of carbon dioxide to remove oxygen from the hemoglobin and produce sickling The film was then made and allowed to dry in a chamber containing the same gas mixture to maintain the hemoglobin in the anoxic form. When a blood film is dried in room air the sickled forms are well oxygenated and revert to a normal shape The sickled forms (*7*) stained with Wright's stain exhibit the great variety of filamentous processes that are seen in unstained wet preparations of blood in which the hemoglobin is reduced by the use of reducing agents such as sodium metabisulfite, ascorbic acid [58] or sodium dithionite [140] or when the oxygen is removed by equilibration with carbon dioxide or by incubation in a sealed wet preparation for 10 to 24 hours. It is interesting to note that the normoblasts (*8*) and the polychromatophilic cells are sickled (*5*)

The platelets appear to be normal in this film and one normal neutrophil (*1*) is seen Obviously the only cells that sickle are those containing hemoglobin of the abnormal variety found in patients with sickle-cell trait or anemia [229]

3 *INTERPRETATION OF BOTH PICTURES* The upper picture is from a blood film from a Negro girl who has maintained a red cell count of 2 0 to 3 5 × 10⁶/mm³ a hemoglobin level of 6.2 to 9 4 gm/100 ml and reticulocytosis during a period of nine years of observation On many occasions an acute crisis with increased anemia and muscle pains has been precipitated by slight infections Otherwise the patient has maintained fairly normal health.

The lower picture is from a Negro male who has maintained a red-cell count of 4 0 to 5 4 × 10^6/mm³ a hemoglobin level of 9 5 to 12 6 gm/100 ml and reticulocytosis during a period of eight years of observation

4 *SICKLE-CELL ANEMIA AND SICKLE-CELL TRAIT* The hereditary nature of the sickling phenomenon was recognized in 1917 by Emmel [88] who observed the occurrence of sickling in a father and son on a sealed coverslip preparation of blood Since then extensive genetic studies have been made which are reported by Neel and

others [6 209 210 211, 212, 262] They showed that the relation between sickle cell anemia and sickle cell trait was due to the presence of the sickling gene as a heterozygote in the trait and as a homozygote in the anemia

Sickle cell anemia is usually evident from the blood values and the presence of sickled forms on the blood film Nucleated red cells polychromatophilic cells, and target cells are usually present When the sickling phenomenon is present as a trait the clinical state and morphologic hematology may be normal In such cases under abnormal physiologic or pathologic conditions the sickling phenomenon may lead to clinical disorders [50 205] In 1927 [114] Hahn and Gillespie showed that sickling is a reversible phenomenon, depending on the degree of oxygenation of the hemoglobin In the homozygous state the erythrocytes contain enough of the abnormal hemoglobin (hemoglobin S) to allow sickling within the physiologic range of oxygen tensions but in heterozygous form the oxygen tension must be abnormally diminished to cause sickling The sequence of events in the pathologic physiology of this disease state is discussed by Harris and coworkers [120] Whenever hemoglobin S is present sickling can be demonstrated by reducing the oxygen tension artificially and thus increasing the percentage of sickled forms and the viscosity [109] The simplest method for the demonstration of sickling is the use of a reducing agent such as 2 percent sodium metabisulfite [58] One or two drops of a freshly prepared solution is mixed with one drop of blood on a slide and covered with a coverglass on which pressure is momentarily exerted to extrude excess blood and produce a thin film This preparation is examined under high-dry power of the microscope immediately and for at least 15 min As many as 80 to 100 per cent of the red cells may show these filamentous sickled forms during this period Sometimes the sickle cells appear more like a holly leaf in shape A positive result indicates the existence of a moiety of hemoglobin S whether in the homozygous or heterozygous state The bisulfite test is the most effective and practical method of effecting deoxygenation and of evoking sickling It is a useful screening test to determine the incidence of sickling among Negro or other populations and for genetic epidemiologic, and ethnic studies [169 213]

5 *HEMOGLOBINOPATHIES* When Pauling and co workers [229] introduced the electrophoretic method of studying hemoglobin a new field was opened up Not only has a better understanding of the sickle cell disease been attained but other abnormal hemoglobins have been observed To date in addition to the normal adult hemoglobin (A) and fetal hemoglobin (F) hemoglobin S, C, D, E, G, H I J and K have been observed [41, 140 262, 302, 316] Hemoglobin S as a heterozygote has been observed in combination with hemoglobin C [215 235] hemoglobin D [153 154 281] and hemoglobin G [258] as well as with the Cooley trait (p 36) Hemoglobin F is present in increased amounts in many of these hemoglobinopathies Some of the morphologic observations are shown in Table 3 Observations on variants of sickle cell disease are discussed by Griggs and Harris [110]

In the homozygous state hemoglobin C is associated with a mild anemia the most striking feature morphologically being the presence of target cells [121, 256 263 271, 284] Target cells are evident in the blood film in the C trait but anemia is absent

Generalizations about the abnormal hemoglobins D, G H I J and K are not possible because of the limited number of cases observed at the present date

/ 13 PLATE VII HEMOLYTIC ANEMIA — CHRONIC AND ACUTE

1 *UPPER PICTURES CHRONIC HEMOLYTIC JAUNDICE — HEREDITARY SPHEROCYTOSIS* (two films from the same sample of blood, the *left hand* film is stained with Wright s stain, the *right hand* film is stained with brilliant cresyl blue and counterstained with Wright s stain)

(a) Description The red cells show moderate variation in size although the average size is about normal This variation in size results from the presence of three types of cells normal red cells (8) spherocytes (9) and large polychromatophilic cells (10) in the left hand and reticulocytes (11) in the right hand pictures The spherocytes are small round and intensely stained and are characteristic of this disease which is also called hereditary spherocytosis The variation in intensity of staining is the striking feature in these two films The larger cells that are polychromatophilic (10) and the reticulocytes (11) are slightly less intensely stained than the normal cells (8) as is often the case with these newly formed cells The spheroidal cells are hyperchromic and because of their shape lack the central pallor of the normal biconcave red cell

Normal white cells are present namely monocytes (6) neutrophils (1) and eosinophils (5) The platelets appear normal

(b) Clinical Discussion These blood films were obtained from a patient with typical congenital hemolytic jaundice who refused splenectomy He has been under observation for 20 years with chronic anemia and reticulocytosis Except for four episodes of biliary obstruction when jaundice was intense with bilirubinuria the patient has had moderate icterus and acholuria His red cell count has varied from 3 0 to $4\ 5 \times 10^6/mm^3$ and his hemoglobin from 7 8 gm to 10 9 gm/100 ml The reticulocytes have fluctuated between 5 and 20 percent The osmotic and mechanical fragilities of the red cells have been increased Similar findings should also be manifest in at least some members of the patient s family

2 *LOWER PICTURE ACUTE HEMOLYTIC ANEMIA — SULFANILAMIDE INTOXICATION* (with leukemoid reaction)

(a) Description The red cells show more variation in size than in the upper drawings Polychromatophilic macrocytes (10) spherocytes (9) and normal cells (8) are present In addition there are irregular forms (13) and nucleated red cells — normoblast (14) and late erythroblast (15) In addition to the appearance of young red cells in this film there are immature white cells of the granulocyte series — band neutrophil (2) metamyelocyte (3) and myelocyte (4) A normal monocyte (6) and a small lymphocyte (7) are also present The platelets are increased in number

(b) Clinical Discussion The lower picture was obtained from the blood film of a 23 year old man who because of scarlet fever was treated with sulfanilamide for only 3 days On the fourth day the patient had marked hemoglobinemia and hemoglobinuria with a decrease in red cell count to $1\ 3 \times 10\ /mm^3$ and in hemoglobin to 4 4 gm/100 ml There was an increase in the white cell count to $54 \times 10^3/mm^3$ with a myeloid response of the granulocytic series and an increase in immature forms of the erythrocytic series appearing in the peripheral blood There was an increase of reticulocytes to 8 4 percent an increased mean corpuscular volume ($111\ \mu^3$) and increase in the osmotic fragility of the red cells The icterus index could not be read because of hemoglobinemia but the bilirubin was elevated as shown in Table 9 The number of nucleated red cells increased to 10 per 100 white cells Howell Jolly bodies and stippled cells though not shown in the plate were present as well as the polychromatophilic cells

The patient recovered promptly after transfusions from this fulminating episode of hemolytic anemia which was characterized by intravascular hemolysis increased osmotic fragility of the red cells and a marked physiologic response of the bone marrow

3 *INTERPRETATION* The blood pictures in these two hemolytic anemias have a great deal in common In both conditions the peripheral blood shows polychromatophilic cells (or when vitally stained reticulocytes) normal cells and spherocytes Both cases had an increased osmotic fragility of the red cells consistent with the presence of spherocytes In one patient there was abnormal icterus (bilirubinemia) in the other hemoglobinemia and hemoglobinuria

The blood picture in the acute hemolytic anemia is more abnormal than in the chronic hemolytic jaundice as evidenced by the presence of immature granulocytes nucleated red cells and the fragmented red cells and half moon shapes Such a striking change in blood picture may also be seen in acute hemolytic anemia resulting from

a variety of causes such as naphthalene poisoning, thermal burns hemolytic transfusion reactions or acquired hemolytic jaundice [55 118 260]

14 PLATE VIII ERYTHROBLASTOSIS FETALIS

1 *DESCRIPTION* The red cells in this film are definitely macrocytic with some variation in size but very little variation in shape The macrocytes whether mature forms or immature forms, are round (compare with the oval macrocytes in pernicious anemia Plate III) The cells are well filled with hemoglobin There are many polychromatophilic cells (*8*) nucleated red cells of all stages (*11–15*) stippled cells (*9*) and Howell Jolly bodies (*10*) Young cells of the granulocytic series (*2 4*) are present in addition to the normal neutrophil (*1*) and lymphocyte (*3*) Platelets appear to be about normal in number and size

2 *INTERPRETATION* This blood film is from a newborn infant who died of erythroblastosis fetalis 16 hours after birth The red cell count was 1 5 × 10⁶/mm³ the hemoglobin 6 2 gm/100 ml The spleen and liver were markedly enlarged The infant s Rh positive red cells were sensitized by anti Rh antibody from the mother In this particular family there was a classic incidence of erythroblastosis fetalis with death of four infants — all but the first child This indicates that the paternal blood group was homozygous Rh positive

This blood picture can be differentiated morphologically from other macrocytic anemias because there is more variation in size here than in any other of the anemias except possibly pernicious anemia The picture is *not* characteristic of pernicious anemia, because the macrocytes are not oval there is comparatively little variation in shape, and there is much more polychromatophilia than is seen in untreated pernicious anemia

The presence of many polychromatophilic cells and nucleated red cells is consistent with the physiologic response of the bone marrow in subacute or chronic hemolytic anemia An acute hemolytic anemia does not usually show such a marked macrocytosis (compare Tables 9 and 10) However, the red cells in the newborn infant are normally macrocytic are well filled with hemoglobin [206] and show moderate polychromatophilia The mean red cell diameter in the normal infant is 8 6 μ The macrocytosis and reticulocytosis or erythroblastosis due to the hemolytic reaction are added therefore to the normal macrocytosis of the infant s blood cells In addition in an infant or child, because of the large volume of active bone marrow, any extra demand on the marrow readily produces immature cells of the erythrocytic and granulocytic series that appear in the peripheral blood

15 PLATE IX ACUTE BLOOD LOSS, CHRONIC LEAD POISONING

1 *UPPER PICTURE ACUTE BLOOD LOSS* (physiologic response of bone marrow)

(*a*) Description The red cells show slightly more than normal variation in size The average size of the new young polychromatophilic cells (*7*) is slightly increased and there are some cells present that are slightly smaller than the normal cells (*4*) there is practically no variation in shape The hemoglobin content of the cells is about normal One target cell is present (*9*) The white cells include a typical neutrophil (*1*) and monocyte (*3*) The platelets (*10*) are about normal in number, but some are large

(*b*) Interpretation This is the blood picture in a young woman 25 years of age ten days after the onset of acute blood loss from menorrhagia There was no evidence of chronic blood loss The polychromatophilic cells (reticulocytes 9 percent Plate IX and Table 11) represent the immature red cells which appeared in the peripheral blood as a normal physiologic response to the acute blood loss in a patient without iron deficiency At

the time when these young red cells are present in increased numbers the mean corpuscular volume is increased (104 μ^3) and the mean diameter of the cells is also greater than normal

The variation in size might be confused with that observed in moderate hypochromic microcytic anemia (Plate IV upper picture) However the red cell indices in this patient show slight macrocytosis and normochromic red cells Also the cells of this blood film when compared with those of a normal blood are seen to be well filled with hemoglobin Hemolytic anemia must be considered because of the presence of polychromatophilic cells the slight macrocytosis and the variation in size The normal icterus index is evidence against increased destruction of red cells but does not exclude it completely The final diagnosis depends upon the recovery from the anemia with or without administration of iron therapy

2 LOWER PICTURE CHRONIC LEAD POISONING

(*a*) Description The red cells in this blood film appear normocytic with slight variation in size but very little variation in shape The hemoglobin concentration of the cells is normochromic The striking abnormality here is the presence of a large number of stippled cells (*8*) and polychromatophilic cells (*7*) Stippling may occur in either the adult red cell or the younger form (polychromatophilic cell) The stippling is observed in a film stained with Wright s stain alone and does not require staining with brilliant cresyl blue Stippling should not be confused with the reticulum of young red cells (see Plate I) which does require brilliant cresyl blue for its demonstration The stippling varies in degree from coarse granules to fine punctate material

The white cells include a normal neutrophil (*1*) and lymphocyte (*2*) The platelets (*10*) appear normal

(*b*) Interpretation Although the presence of stippled cells alone does not necessarily indicate chronic lead poisoning the occurrence of stippled cells and polychromatophilic cells associated with normal or slightly hypochromic red cells does suggest lead poisoning According to the data in Table 11 there is a moderate normocytic anemia with increased reticulocytes (8 percent) and a normal icterus index Accordingly the large number of stippled cells which represent 2 8 percent of the red cells serves as a clue to distinguish this case from that of acute blood loss (upper picture) or hemolytic anemia Stippled cells however are also characteristic of Cooley s trait and of many other anemias but usually are fewer in number and are associated with other changes in the red cells The diagnosis of chronic lead poisoning may be confirmed by the history of exposure to lead and by demonstrating an abnormal excretion of lead and coproporphyrin in the urine [116 297] Since the type of porphyrin excreted in lead poisoning is abnormal it is possible that the stippling of the red cells is an expression of a disturbance in metabolism or retardation of the formation of hemoglobin [297] The anemia does not respond to the usual antianemia therapy

16 PLATE X LEUKOCYTOSIS

1 DESCRIPTION OF BOTH PICTURES UPPER PICTURE Leukocytosis associated with carcinoma of the lung *LOWER PICTURE* Leukocytosis with toxic manifestation due to overwhelming sepsis

In both of these patients the peripheral blood showed a definite leukocytosis increase in the count being due to the cells of the granulocytic series The predominating cell is the young neutrophil or band form (*6*) In the pictures there are only two adult neutrophils (*1*) present the essential requirement for the adult form being two or more lobes of the nucleus connected by a thread In these pictures younger cells of the granulocytic series are also present metamyelocyte (*3*) myelocyte C (*4*) and myelocyte B (*5*) These bands and metamyelocytes which are often larger in infectious conditions than in normal blood should be compared with the monocytes (*8*) for contrast in staining reactions and structure The difference between the lacy structure of the nucleus in the monocytes (*8*) and the homogeneous chromatin of the myelocytes is very well shown as is also the difference in the inten

sity of the blue of the cytoplasm The young neutrophils or bands (*2*) and the metamyelocytes (*3*) in the lower picture show large toxic granules and marked vacuolization of the cytoplasm One of the cells (*2a*) shows a Dohle body A plasma cell (*10*) and a histiocyte (*9*) are present as well as lymphocytes (*6 7*) In the case shown in the upper picture the white cell count was 35 000/mm³, with 95 percent of the cells in the granulocytic series In the case shown in the lower picture, the white cell count was 29 000/mm³ although in such a toxic condition the white cell count may be depressed The red cells appear normochromic in both cases, and they are slightly macrocytic in the lower picture The platelets appear increased in the upper picture, as is usual with a normal response of the bone marrow they appear to be decreased in the lower picture a fact which has a grave prognosis in infection

2 *INTERPRETATION* The granulocytic response as shown in the upper picture is typical of a physiologic response to exercise mild infection especially with pyogenic organisms acute blood loss acute hemolytic anemia convulsions infarcts thrombosis diabetic acidosis uremia, certain drug intoxications carcinoma tissue necrosis polycythemia vera and after splenectomy

The granulocytic response as seen in the lower picture is much more severe and abnormal This is a picture of overwhelming sepsis and indicates a grave prognosis There are no adult neutrophils in the picture An increase in young forms has been termed a shift to the left by Arneth [9] Schilling [254] and others but actually it means a shift to immaturity with a reduction of the marrow threshold with liberation of increased numbers of young forms Many classifications and hemograms have been used to interpret the meaning of the changes in the maturity of the cells It is essential to differentiate the mature forms of granulocytes (cells in which the nucleus has two or more lobes connected by a thread) from the immature forms with band indented or round nucleus (bands metamyelocytes and myelocytes) This increase of immature cells may be termed a regenerative shift which is the normal physiologic response, or a degenerative shift in cases in which there is a depression of the bone marrow with an insufficient supply of the neutrophils in the peripheral blood to meet the needs The degree of immaturity indicates the severity of the reaction In severe reactions when myelocytes and immature red cells appear in the peripheral blood the term myeloid response is used Leukemoid responses have been reported in septicemia [160], in chronic and acute infections [81, 275], acute hemolytic anemia [124 129 287] and in acute blood loss [57] The presence of heavy blue black granules in the neutrophils bands, and metamyelocytes as seen in the lower picture is an indication of severe infection or necrosis [108 162] These toxic granules are usually present in pyogenic infections certain viral infections necrosis malignant tumors and liver disease Marked vacuolization as seen in some of the cells in the lower picture, is usually considered to be a stage in the disintegration of the cells This is often seen in the cells in overwhelming infections This vacuolization should not be confused with that present in the cells when the films are made from blood preserved with an anticoagulant Dohle bodies were described by Hill [130] in cases of streptococcal infections, but subsequent observations have shown that they may be present in other types of severe infections The qualitative changes in the white cells are fully as important in interpreting the blood picture as the quantitative changes Plasma cells (*10*) and histiocytes (*9*) are frequently seen in response to increased demand on the blood forming tissue

3 *LEUKOCYTOSIS IN INFANTS AND CHILDREN* The normal baby at birth may have a leukocytosis of 10 0 to 45 0 × 10³/mm³ This white cell count decreases during the first 26 weeks Children may have a white count slightly greater than adults Osgood and associates [220] in a study of 86 children from 4 to 7 years of age found a mean value for white cells of 10 419 ± 0 176 × 10³/mm³, with 95 percent of the observations showing a range of 5 5 to 15 5 × 10³/mm³ The bone marrow in infants and children is more active than in the adult Therefore for a given response the increase in the white count is greater and the presence of immature granulocytes and erythrocytes in the peripheral blood does not imply as serious a prognosis as it would in an adult A myeloid reaction in an in

fant with pneumonia was reported by Buckman [33] in which the white cell count rose to 84 5 × 10^3/mm^3 with 9 percent myelocytes There were nucleated red cells and megakaryocyte fragments in the peripheral blood

4 *LEUKOPENIA* The mechanism of leukopenia may involve decreased production by the bone marrow redistribution after delivery to the circulation or increased loss or destruction of the cells The total white cell count may be decreased with a decrease of neutrophils either relative or absolute The neutrophils that are present are usually young forms In certain conditions the neutrophils may be decreased in absolute number with a relative increase of band forms and perhaps metamyelocytes Such changes have been seen in bacterial endocarditis miliary tuberculosis and paroxysmal nocturnal hemoglobinuria Neutropenia may be due to depression of the bone marrow as the result of toxic action of or hypersensitivity to certain drugs and chemicals such as benzol amidopyrine dinitrophenol the sulfonamides thiouracil and chloramphenicol [46 155 164 171 295] Neutropenia may be the result of replacement of the normal marrow with foreign cells as in metastatic carcinoma xanthomatosis or fibrous tissue Neutropenia with a relative lymphocytosis is usually seen in pernicious anemia and in certain other conditions where there is a known nutritional deficiency Neutropenia is the usual response of the bone marrow to anaphylactic shock The association of splenomegaly and sometimes hepatomegaly with neutropenia has been considered by Wiseman and Doan [309] as a separate entity called primary splenic neutropenia Cyclic neutropenia has also been reported [52 222 247]

5 *AGRANULOCYTOSIS* This disorder is characterized by an acute course fever necrotic lesions of the mucous membranes leukopenia and severe granulocytopenia In such cases the platelets and red cells are usually normal with the striking changes affecting only the granulocytic series The association of agranulocytosis with sensitivity to drugs has been demonstrated by many authors [68 144 181] A case of agranulocytosis was reported by Jackson *et al* [143] in which there was a periodic decrease in the neutrophils associated with the menstrual cycle More recent work has demonstrated an immunologic agranulocytosis [70 198]

6 *EOSINOPHILIA* An increase in white cell count with an absolute increase of eosinophils occurs in allergic diseases parasitic infestations skin diseases neoplastic disease periarteritis nodosa and Loeffler s syndrome and during the recovery phase in many infections It is interesting that multilobed eosinophils are often seen in these responses of the bone marrow while the eosinophil with a bilobed nucleus is usually seen in the normal blood [57 253]

7 *EOSINOPENIA* It has been well recognized clinically that an early sign of severe infection especially by pyogenic organisms is the disappearance of the eosinophils from the peripheral blood conversely a sign of recovery is their reappearance [242 303] Eosinopenia is considered a regular manifestation in such conditions as shock major surgical procedures and Cushing s disease and following the injection of certain adrenal cortical hormones [131 289]

8 *BASOPHILS* The number of basophils in the peripheral blood is low (0 to 2 percent) therefore there is no clinical significance to the absence of basophils However they usually disappear during severe infection and reappear with recovery as do the eosinophils Increase of basophils is a frequent finding in myelogenous leukemia myeloid metaplasia and polycythemia vera In leukemia the relative number of basophils may be exceedingly high following radiation but the absolute number may be the same as the pretreatment level

9 *HEREDITARY ANOMALIES OF THE WHITE BLOOD CELLS*

(a) Pelger Huet Familial Anomaly In this condition first described by Pelger the neutrophils of the peripheral blood have bilobed nuclei while the number of band forms is increased Metamyelocytes and myelocytes are occasionally seen The chromatin structure of the nucleus of the neutrophils appears clumped as in a mature cell while the nuclear form is immature Individuals with this abnormality are clinically normal and respond to infection and other demands on the bone marrow with an increase of immature granulocytes Huet in 1932 [133] produced evidence that this anomaly occurred in families

Since then the dominant hereditary nature has been confirmed by other investigators [20, 159, 290]

(*b*) Hereditary Granulation Anomaly Alder [5] first described the occurrence of abnormal coarse blue black granules in the neutrophils of individuals who had no infection or known disease This abnormal granulation was present in the neutrophils and eosinophils as well as in the younger cells of the granulocytic series This condition was observed by Alder repeatedly in one woman over a period of 8 years and in two members of another family Jordans [152] has studied a family in which this granulation anomaly was observed in many members of one family representing three generations

(*c*) Familial Eosinophilia Several reports [7, 278] may be found in the literature in which *an increase of eosinophils was present in several* members of a family When no other cause for the eosinophilia is found, this is considered a familial anomaly

17 PLATE XI MYELOGENOUS LEUKEMIA

1 *DESCRIPTION OF BOTH PICTURES* The pictures represent blood films from two patients with chronic myelogenous leukemia

(*a*) White Cells By a study of these plates, practically all the typical cells in the development of the granulocytic series can be identified In the upper picture there are two neutrophils (*1*) and two young neutrophils or band forms (*3*) One of these band forms is much younger than the other and by some might be classed as a metamyelocyte The nucleus however has two lobes connected by a band like a dumbbell The character of the nucleus is mature with clumped chromatin A typical eosinophil (*2*) is shown with large discrete red orange granules Three basophilic cells (basophils) are present — a young basophil (*5*) an abnormal form (*6*) and a basophilic myelocyte (*12*) The basophilic granules in the myelocyte B (*12*) *should be contrasted with* the neutrophilic granules of myelocyte B (*10*) The dark blue basophilic granules are definitely larger than those of the neutrophilic myelocyte (*10*) but are usually smaller than the basophilic granules in the more mature basophils (*5* and *6*) Eosinophilic myelocytes (*11*) are striking because of the large red orange granules which cover the round nucleus The granules in one of the eosinophilic myelocytes do not have their full color although they are large (In other patients with myelogenous leukemia the eosinophilic myelocytes frequently contain granules that are smaller *than those shown in* Plate XI Occasionally both eosinophilic and basophilic granules occur in the same cell) There is considerable contrast between the eosinophilic myelocyte B (*11*) and the more mature forms — the eosinophilic myelocyte C (*9*) young eosinophil (*4*), and adult eosinophil (*2*)

In the lower picture all stages of the neutrophilic series of granulocytes are shown The typical myeloblast (*14*) is shown with a fine granular nucleus containing a nucleolus and surrounded by a rim of deeply basophilic cytoplasm, without granules A similar cell (*13*) with a typical blast nucleus and basophilic cytoplasm but containing nonspecific, azurophilic granules is called a myelocyte A or early myelocyte By many observers this could be considered a blast because of the nucleolus The granules in the cytoplasm are not differentiated into neutrophilic basophilic and eosinphilic types until the cell is at a more mature stage (myelocyte B) The myelocyte B *of all types has already been described* (*10 11 12*) The myelocyte C, containing neutrophilic granules (*8*) has the same type of nucleus and cytoplasm as the neutrophilic myelocytes of stage B (*10*) However, the granules which are scattered throughout the cytoplasm do not cover the nucleus The homogeneous nuclear structure and the light gray blue color of the cytoplasm are the same in both the myelocyte B and the myelocyte C Occasionally, the granules in the myelocyte C are absent or poorly stained although this stage of development may be identified by comparing it with the myelocyte B of the same film The metamyelocyte (*7*) should not be confused with the myelocyte C (*8*) since the cytoplasm of the metamyelocyte has acquired the

pink lavender color of the neutrophil with a nucleus that is beginning to show slight condensation of chromatin This metamyelocyte may be compared with the band form and adult neutrophil in the same film for the color of the cytoplasm In the young neutrophil (*3*) the two lobes of the nucleus are distinct but connected by a thick band whereas in the adult form of the neutrophil the lobes of the nucleus are separated by threads of chromatin

The young form of the monocytic series (*15*) shown in the upper picture is often confused with the metamyelocyte (*7*) or the late myelocyte (*8*) However the young monocyte with a round nucleus has the same lacy or reticular structure of the nucleus and often the same folded or fissured outline of the nuclear chromatin as in the lobulated nucleus of the mature monocyte (see Plate II *10*) These cells may best be differentiated by comparison with known cells of the same series in the same film The young monocyte should be compared with the adult monocyte for size nuclear structure color of the cytoplasm and type of granulation The myelocyte C should be compared with the myelocyte B of the same film The nuclear structure of these cells as shown in Plate XI is quite different Thus the monocyte has a reticular, fissured nucleus the metamyelocyte shows the first stages in the condensation of the chromatin that occurs as the cell matures whereas the myelocyte C has a homogeneous nucleus Similarly the cytoplasm of each of the three varieties of cells should be compared with that of the others and of related cells of the same series Monocytes and lymphocytes are usually present in the blood film in myelogenous leukemia but the relative number is small since the increase is due to cells of the granulocytic series

(*b*) Red Cells and Platelets The red cells show some variation in size and intensity of staining A normoblast (*17*) and late erythroblast (*18*) are present in the lower film In both cases the platelets appear to be increased There are masses of platelets (*19*) in association with a megakaryocyte nucleus (*20*) The megakaryocyte nucleus (*20*) shows a typical dark rim surrounding masses of chromatin appearing as a wavy structure

2 *INTERPRETATION* There is little doubt about the diagnosis when blood films such as those shown in Plate XI are seen The obvious diagno is is myelogenous leukemia becau e both films are characterized by an extreme increase in the white cell count and immaturity of the granulocytic series of cells representing all stages The changes are an order of magnitude greater than occurs in nonleukemic conditions The abnormalities of the red cells and platelets are also consistent with the complications of leukemia — replacement of the marrow with leukemic tissue The prognosis of a given case can be estimated in part by an appreciation of the degree of abnormalities in the film

(*a*) Upper Picture Chronic Myelogenous Leukemia In the case with this blood picture the prognosis is consistent with a chronic process and the patient may be aided considerably by treatment This prognosis is indicated by the maturity of the granulocytic series The youngest cells present are the stage of myelocyte B The presence of basophilic cells in increased numbers is of interest since basophilic myelocytes are frequently increased in untreated chronic myelogenous leukemia They may also be relatively increased after radiation therapy although the absolute number is probably no greater than the pretreatment level [57] After prolonged radiation therapy the cells become more and more abortive This is especially true in the basophils in chronic myelogenous leukemia Cases in which many basophilic cells have been observed throughout the disease have been called basophilic leukemia [38 76] The red cells in this blood film seem quite normal in appearance but the patient has a significant anemia (see Table 13) The platelets are increased and located in the vicinity of a nuclear fragment of a megakaryocyte These are frequently seen in the peripheral blood in chronic myelogenous leukemia [193 195]

(*b*) Lower Picture Advanced Chronic Myelogenous Leukemia The prognosis of the patient whose blood film is repre ented in the lower picture is consistent with an advanced stage of chronic myelogenous leukemia This is indicated by the marked degree of immaturity of the granulocytic series which is represented in this instance by the presence of myeloblasts and by the presence of nucleated red cells The platelets are

markedly increased A marked increase or a marked decrease in the number of platelets indicates extensive involvement of the bone marrow and is consistent with a bad prognosis

(*c*) ACUTE MYELOGENOUS LEUKEMIA Some cases of leukemia yield a more immature blood film than that shown in either of these pictures When the predominating cells in the peripheral blood are myeloblasts the leukemia is considered to be acute or the terminal phase of a chronic leukemia The myeloblasts in such acute leukemias may be present in many abnormal forms Some myelocytes may be present with the myeloblasts *an observation which may aid in the identification* of the blast form as a myeloblast Some nucleated red cells are usually present in acute leukemia The platelets may be markedly decreased in number large and abnormal in appearance Anemia or hemorrhage may be a major cause of death

(*d*) SUBLEUKEMIC OR ALEUKEMIC LEUKEMIA *If the white cell count were low then the interpretation* of these blood films would be more difficult Patients with leukopenia or normal white cell counts but with myelocytes or myeloblasts and often nucleated red cells in the blood may be said to have subleukemic myelogenous leukemia In instances of aleukemic myelogenous leukemia there may be no or only a few ab*normal forms of the granulocytic series in* the peripheral blood and a low or normal white cell count but there may be nucleated red cells and thrombocytopenia In these instances of subleukemic or aleukemic leukemia the diagnosis may be confirmed by bone marrow puncture or by observing the development of the disease which may become manifest as leukemia as it advances

(*e*) AGNOGENIC MYELOID METAPLASIA OF THE SPLEEN [147] (Chronic Nonleukemic Myelosis [37 128] Leuko erythroblastic Anemia with Myelosclerosis [*294*] *Myelofibrosis* [*159*]) These terms are most frequently used although in a recent review of the literature Block and Jacobson [25] have mentioned some 25 other designations for this syndrome In agnogenic myeloid metaplasia, active blood formation takes place in the spleen liver, or other extramedullary sites This condition is most easily confused with mye*logenous leukemia and* the importance of differentiating these two diseases has been pointed out by Jackson Parker and Lemon [147] by Hickling [128] and by Block and Jacobson [25] Myeloid metaplasia may be associated with exposure to industrial solvents such as benzol, and *may be preceded by polycythemia* [*236*] For example, of 15 patients with erythremia reported by Minot and Buckman [194] three developed anemia and a leukemoid blood picture The sections of the spleen of one of these have been reviewed and showed changes consistent with agnogenic myeloid metaplasia The description of the blood picture in the cases described by Minot and Buckman [*194*] *and their accompanying plate give an* accurate reproduction of the changes in the red and white cells seen in the polycythemic phase of agnogenic myeloid metaplasia The clinical picture and the hematologic findings vary markedly especially as the disease progresses The essential physical finding is progressive and extreme enlargement of the spleen without lymphadenopathy

The blood film is characterized by marked variation in size and especially in the shape of the red cells with nucleated red cells The presence of nucleated red cells occurring when the red cell count is 4×10 /mm³ or as high as 6 or 7×10^6/mm³ in cases of polycythemia is a finding of diagnostic importance In myelogenous leukemia the red cell changes early in the disease usually are not so great as in myeloid metaplasia In myeloid metaplasia the red cells showed tailed forms microcytes and many bizarre forms that are more characteristic of myelophthisic anemia than of any other anemia Stippled cells Cabot ring forms Howell Jolly bodies and polychromatophilic cells are found as the disease progresses Continuing reticulocytosis is characteristic with occasional cases in the anemic phase showing values as high as 30 percent A few cases have *been observed in which an overwhelming hemolyt*ic anemia has been present [31 147]

The presence of immature cells of the granulocytic series occurring when the white cell count is low or normal and the red cell count is normal or increased is also characteristic As the disease progresses more immature cells appear and in some cases the white-cell count may increase to 200×10^3/mm³ making the clinical differentia

tion from myelogenous leukemia difficult or impossible The condition may occasionally terminate in leukemia The disease may be one of prolonged chronicity (20 years) with extreme splenomegaly and with the blood picture described above In some cases the platelets have been increased as in myelogenous leukemia associated with fragments of megakaryocyte nuclei in the film Platelets may be large and extremely bizarre In other ca es thrombocytopenic purpura may be the presenting symptom Splenectomy may be fatal since active blood formation is tak ing place in the pleen although in some instances patients have lived many years [57 239] following removal of the spleen

(*f*) MYELOPHTHISIC ANEMIA In carcinoma arising in the breast prostate thyroid stomach or kidney the presence of myelocytes myeloblasts and nucleated red cells in the peripheral blood may be the first evidence that metastasis to the bone marrow has occurred [124 142 149 288] In carcinomatosis there may be a high white cell count an increase of myelocytes occasional myeloblasts and toxic granulation of the neutrophils A similar leukemoid response of the myeloid type may be seen in disseminated tuberculosis [142 287] or when the bone marrow i invaded by lymphoma or leukemic tissue The pre ence of nucleated red cells when the red cell count is above 4 × 10 /mm and of abnormal cells of the granulocytic series when the white cell count is normal or slightly increased should be considered as possible signs of invasion of the bone marrow

18 PLATE XII MONOCYTIC LEUKEMIA

1 *DESCRIPTION OF UPPER PICTURE* The predominating cells in this film are large cells with gray blue cytoplasm finely peppered with granules that are concentrated around the periphery The granules are usually blue purple but are sometimes azurophilic The e cells are different stages of the monocytic series As de cribed previously (p 22) the nucleus of the monocyte is somewhat irregular in outline is not sharply defined as is the lymphocyte nucleus and has a lacy reticular chromatin structure which may have a folded or grooved appearance As seen in this picture this lobulation is apparent in the adult monocyte (*6*) in the young monocyte (*7*) and even in the blast (*8*) The nucleus of the mature monocyte (*6*) is indented and the lobes are more striking than in the cells classed as young forms (*7*) which have a round nucleus Thi may also be observed in Plate II in which the monocytes have a typical horseshoe shape These characteristics should permit differentiation of the monocytic series from the pink lavender cytoplasm and clumped chromatin nucleus of the neutrophil (*1*) and band form (*2*) The monocyte is nearly always much larger than the neutrophil In infectious states abnormally large band forms may be confused with the monocyte In such instances the characteristics of the known cells must be compared with those of the unknown The small lymphocyte (*3*) with clear light blue cytoplasm and sharply defined nucleus with condensed chromatin would not be confu ed with the monocytic series as shown in this film The red cells are quite uniform in size and shape but are slightly macrocytic. The platelets are slightly decreased

2 *DESCRIPTION OF LOWER PICTURE* In this film the predominating cells are young monocytes (*7*) and blasts (*8*) These are considered monoblasts because they have the same type of folded nucleus as is present in the young and adult monocytes The three monoblasts (*8a*) with nucleoli represent the youngest cells The nucleoli are sharply defined areas in the nucleus that take a lighter stain The nuclear chromatin is slightly more reticular in these blasts than in the myeloblast (Plate XI) or the lymphoblast (Plate XIV) Azurophilic granules may be present in the monoblast and may represent a stage of maturation comparable to the myelocyte A stage in Fig 4 Azurophilic rods or Auer bodies are present in the cytoplasm of some of these monoblasts (*8b*) It should not be difficult to distinguish the myelocyte with its homogeneous nucleus sometimes covered with granules (*4*) from the monocyte with its loose reticular nucleus The histio-

cyte (5) in the lower right hand corner should be observed with care since this cell is often incorrectly classed as a monocyte The histiocyte is characterized by the irregular contour of the cell the spongy, vacuolated cytoplasm which may or may not show active phagocytosis and the honeycomb structure of the nucleus The red cells show moderate variation in size The platelets are definitely reduced as occurs in most acute leukemias

3 INTERPRETATIONS

(a) Resume of Cases These blood films are from two patients with monocytic leukemia (A47 30 and A45 310 Mallory Institute of Pathology Boston City Hospital) The patient whose blood film is shown in the upper picture was a 73 year old woman who entered the hospital because of anemia (red cell count 1 4 $\times 10^6/mm^3$ hemoglobin 5 3 gm/100 ml) She had noted progressive weakness and exertional dyspnea for approximately 1 year Her diet had been markedly restricted in variety and amount There were two previous admissions 7 and 12 years before with diagnoses of psychoneurosis malnutrition and vitamin deficiency Aspiration of the bone marrow on her last admission showed hyperactivity of granulocytic monocytic and erythrocytic series with extremely young red cells She was first considered to have a nutritional macrocytic anemia and was given liver extract but her anemia did not respond to therapy Her white cell count on admission was 7 1 $\times 10^3/mm^3$ with 34 percent of monocytes The white cell count increased steadily while under observation to $300 \times 10^3/mm^3$ and the proportion of monocytes increased to 92 percent Throughout the period of observation the characteristic cells were the large monocytes with gray blue cytoplasm containing fine scattered blue purple granules and typical lobulated nuclei with the loose lacy structure Young monocytes and monoblasts were present but the mature forms predominated The duration of the disease after the diagnosis of leukemia became manifest was only 1 month Although her presenting symptoms at this admission were those of anemia presumably related to the leukemia it is impossible to judge how long the leukemic process had been active

The blood film shown in the lower picture was from a 41 year old man with severe anemia thrombocytopenia and high white-cell counts The clinical manifestations at this time were typical of an acute leukemia with a sudden onset 5 days before admission symptoms of a mild upper respiratory infection and slight sore throat headache and pain in the arms and legs and back The patient had nosebleeds petechiae gastro intestinal hemorrhage and bleeding from a rectal fistula An ischiorectal abscess had been operated upon 5 months previously but it had not healed properly no data on the blood film at that time are available The patient had a rapid downhill course death occurring after 20 days in the hospital In contrast to the other case the white blood cells were chiefly monoblasts and young monocytes

(b) Prognosis in Monocytic Leukemia. It is difficult to distinguish between acute monocytic leukemia and the terminal phase of chronic monocytic leukemia Patients have been observed who have shown an increased count of monocyt largely adult forms over a period of ma months This has been followed subsequently b clinical manifestations of leukemia and the ap pearance of increased numbers of young monocytes and monoblasts in the peripheral blood during a terminal leukemic period of 2 months [21] Chronic monocytic leukemia occurs although definite diagnosis often is not established until the patient has an acute fulminating infection or hemorrhagic manifestations associated with an acute phase of the monocytic leukemia [99] When the peripheral blood contains monoblasts the prognosis is poor However patients have been observed with mature monocytes in their peripheral blood associated with the symptoms and signs of an acute type of leukemia with gum lesions and hemorrhagic phenomena The duration of life is influenced in part by the control of the infection with drugs and antibiotics

(c) White Cell Count in Monocytic Leukemia The white cell count varies widely from normal values up to several hundred thousand per cubic millimeter The predominating cell is the monocyte A given case may show mature forms as the predominating cell monoblasts as the characteristic form or in most instances different stages in maturation of the monocyte As the dis

ease progresses the type of monocyte may become more and more immature Young neutrophils and myelocytes may also be seen but rarely in numbers great enough to cause confusion Myelocytes may be present in any leukemia or anemia

(*d*) Auer Bodies The Auer bodies shown in some of these cells (*8b*) are characteristically seen in acute leukemias [3] Although they were originally described as occurring in lymphocytes [10] with a better differentiation of the blood cells it is now recognized that they occur in myeloblasts [244] in monoblasts in many cases of monocytic leukemia in histiocytes or reticulum cells and in plasma cells [57 99 107 1,0] According to Leitner [1,0] these Auer bodies are crystal of abnormal protein

(*e*) Anemia A *normocytic* anemia (rarely macrocytic as in these two cases) of moderate to severe degree is usually present by the time a diagnosis of monocytic leukemia can be made Nucleated red cells are often present as one of the characteristics of a myelophthisic anemia

(*f*) Platelets The platelet count is usually decreased This may be manifest occasionally as a presenting complaint by hemorrhagic phenomena a was observed in the patient whose blood film is shown in the lower picture

4 *MONOCYTIC LEUKEMIA DIFFERENTIATED FROM OTHER LEUKEMIAS* The separation of monocytic leukemia from other leukemias was first described by Reschad and Schilling Torgau in 1913 [241] The hypothesis of Ehrlich [85] that the monocyte was a transition form between the myelocyte and the neutrophil impeded progress in the understanding of this cell Naegeli [207] and Evans [90] concluded that the monocyte was an independent cell type of myeloid origin Still further confusion has resulted because the monocyte has not been differentiated by many observers from the histiocyte o reticulum cell The theories of origin of the monocyte have been confusing Forkner [99] for example cites 16 such theories

Clough [47] reported 72 cases of monocytic leukemia from the literature and one of his own but did not differentiate the monocyte from the histiocyte Forkner [99] differentiated acute monocytic leukemia from acute lymphocytic and acute myelogenous leukemia Other reviews of monocytic leukemia are given by Osgood [219] and by Evans [91]

In the majority of instances monocytic leukemia need not be confused with either the myelogenous leukemia or the histiocytic leukemia if the type of cell is studied Although small numbers of myelocytes may be present in a monocytic leukemia and occasionally there appears to be a shower of monocytes in a myelogenous leukemia these two leukemias are readily distinguishable It is necessary to evaluate the blood by making more than one observation For example Hall and Watkins [115] report a case changing from myelogenous to monocytic leukemia probably indicating an early myeloid response in a patient with underlying monocytic leukemia

Watkins and Hall [79 296] have shown that the Schilling type of monocytic leukemia can be distinguished from the Naegeli form by the morphologic characteristics of the predominant cell and its progenitors and by the histopathologic findings in the hematopoietic tissues The Schilling type of monocytic leukemia appears to be a separate entity and is called by some leukemic reticulo-endotheliosis (or histiocytic leukemia) A series of leukemia cases have been studied by Belding Daland and Parker [21] in which differentiation of histiocytic leukemia from the monocytic leukemia has been possible by hematologic pathologic and morphologic studies

5 *MONOCYTIC RESPONSE — MONOCYTOSIS* The evaluation of the literature in regard to monocytic re ponses mu t also be carefully considered with respect to the type of cell described The response of histiocytes and clasmatocytes has been termed a mononuclear reaction throughout the literature but is discussed separately (Sec 19) since these are not cells of the monocytic serie

Monocytes are often increased during the recovery phase of agranulocytosis They are also increased following acute infection and in pneumonia during the period of resolution of the pulmonary process in the acute phase of rheumatic fever [57] in some instances of acute inflammation [100] and in many patients in the active phase of tuberculosis [23 18,] Al o the increase in the number of monocytes was found to be of diagnostic importance in electing before clinical

picture of a blood film was from a patient with histiocytic leukemia one of the series published by Belding and associates [21] in which a clinical summary of this patient (Case 4) and a chart showing the hematologic findings during the course of the disease (Fig 4) are given

The diagnosis of histiocytic leukemia is first suggested by the appearance in the peripheral blood of large irregular cells which do not conform to the characteristics of the granulocytic, monocytic lymphocytic or plasma cell series This may best be seen by comparing the cells in Plate XIII with the cells of the granulocytic series in Plate XI the monocytic series in Plate XII the lymphocytic series in Plate XIV and the plasma-cell series in Plate XV Not only are these cells different from those of the regular series, but the type of cell varies markedly in a given blood sample and is quite distinctive in different cases The variation in size and nuclear structure is quite obvious in Plate XIII In this picture the contrast between the lymphocytes monocytes and neutrophils is striking In this particular case the cytoplasm is deeply basophilic but the structure of the nucleus is not granular like a blast and does not show nucleoli Phagocytosis of red cells as shown in Plate XIII was seen in the peripheral blood in the bone marrow aspirations and in the fixed tissues In some cases the cells may have a characteristic blast nucleus with granular chromatin and nucleoli but still have a light cytoplasm which is not as basophilic as would be expected in a blast The cells are more often confused with lymphocytes or blasts than with monocytes The histiocytes as well as lymphocytes and blasts show a negative peroxidase reaction while the monocytes are usually peroxidase positive as are all the cells of the granulocytic series with the exception of the blasts

The white cell count in the blood film shown in Plate XIII is high ($28.9 \times 10^3/mm^3$) but many of the cases studied [21] have shown a normal or low count When the white cell count is below $3.0 \times 10^3/mm^3$ differential cell counts are more difficult and the few abnormal forms may easily be classified as atypical lymphocytes However these irregular basophilic cells may be the important cells which give the clue that the diagnosis may be histiocytic leukemia. When the white-cell count is low, a buffy coat of the white cells should be studied for the identification of the blast cells Bone marrow aspirations should also be obtained in such cases A biopsy of the bone marrow lymph node or skin lesions which are often present should be made for confirmation of the diagnosis

(*c*) Histiocytic Leukemia in the Literature The literature on histiocytic leukemia is confused because many of the early investigators failed to distinguish the histiocyte from the monocyte Downey [79 80] differentiated the Schilling type of monocytic leukemia (histiocytic) from the Naegeli type of monocytic leukemia (monocytic) Later, Montgomery and Watkins [200] and Watkins and Hall [296] reviewed some of the clinical and hematologic differences between these two types of leukemia There are many case reports of histiocytic leukemia in the literature but this condition has been designated by a variety of terms such as histioleukemia [199] reticulosis [138] aleukemic reticulosis [64] 'leukemic reticulo-endotheliosis" [80 97] and clasmatocytic leukemia [103] in addition there are those cases which are included within the broad term 'monocytic leukemia [63 72]

(*d*) Histiocytes in Reticulum Cell Sarcoma and Hodgkin s Sarcoma (Fig 8 3 and 5) Although the blood findings in Hodgkin s disease are not considered to be diagnostic [145] there are cases of Hodgkin s sarcoma and reticulum-cell sarcoma in which both the ameboid or phagocytic type of histiocyte and the basophilic or leukemic histiocyte have been seen in the peripheral blood. There may be leukopenia normal white-cell count or increased white-cell count due to the increase of abnormal cells (leukemic histiocytes) The type of cell seen in some of these cases is quite similar to those in histiocytic leukemia (Plate XIII) Such a patient (P H 19588) in whom a leukemic blood picture was present during the last hospital admission the white-cell count varying from 4 0 to $2.0 \times 10^3/mm^3$ was reported by Merrill and Jackson [189] The abnormal cells which varied from 2 to 36 percent were large irregular forms with deep blue cytoplasm and nuclei of homogeneous chromatin with nu

cleoli These were considered to be histiocytes Other cases have been observed with histiocytes of various types appearing repeatedly in the film of the peripheral blood [57] These may be seen more readily if the blood films are obtained from the ear lobe as the large cells are often more concentrated in this area in which the capillary circulation may be rather sluggish [59] When abnormal cells of this type appear in the peripheral blood the bone marrow aspiration may show masses of these leukemic histiocytes Biopsy of the lymph nodes skin lesions if present or bone marrow is usually indicated to confirm the diagnosis in such cases

In the review by Dameshek [64] a relation between aleukemic reticulosis and Hodgkin s disease was suggested Marchal and Bargeton [184] report that Hodgkin s sarcoma may show a terminal monocytosis these monocytes are presumably histiocytes

20 PLATE XIV LYMPHOCYTIC LEUKEMIA

1 *UPPER PICTURE CHRONIC LYMPHOCYTIC LEUKEMIA*

(*a*) DESCRIPTION The predominating cell in this film is the mature lymphocyte with its clear blue cytoplasm The nucleus of the lymphocyte (*3*) is rather sharply defined and contains densely clumped chromatin whereas the nucleus in the monocyte (*2*) is irregular sometimes horseshoe-shaped and has a delicate lacy structure or fine network.

The mature lymphocyte is divided into two types according to size The small lymphocyte (*3*) has a nucleus about the size of a red cell and a relatively small amount of cytoplasm The large lymphocyte (*4*) has a nucleus larger than the red cell and usually has abundant cytoplasm The structure of the nucleus is similar to that of the small lymphocyte but often is not so densely clumped The cytoplasm is a clear blue the lightest blue of any of the blood cells and occasionally contains a few scattered red purple granules, and rarely some blue granules

Broken cells (*7*) are common in films of leukemic blood and are not included in the differential count of white cells because the cell type cannot usually be identified The red cells show slightly more than normal variation in size The number of platelets is slightly decreased

(*b*) INTERPRETATION This picture is characteristic of the blood film seen in chronic lymphocytic leukemia Here the predominating cell is the small lymphocyte (*3*) (see p 19) Sometimes the nuclear chromatin is more densely clumped than normal giving the appearance of plasma cells Such cells can usually be differentiated from plasma cells by the fact that the lymphocyte cytoplasm is a clear light blue compared to the green blue of the plasma cell (see Plate XV) Occasionally vacuoles or fenestrations of the nucleus will simulate nucleoli but true nucleoli are usually seen in young forms with deep basophilic cytoplasm The nucleus is usually round although certain cases of leukemia have been observed in which the nuclei have deep indentations [57]

The degree of anemia varies from none to severe myelophthisic forms with variable degrees of thrombocytopenia

Chronic lymphocytic leukemia may be discovered by accident and the patient may live many years Accordingly the identification of mature lymphocytes by careful study of the blood film is an aid in establishing the prognosis of the patient

2 *LOWER PICTURE ACUTE LYMPHOCYTIC LEUKEMIA*

(*a*) DESCRIPTION The cells in this film are more immature than those shown in the upper picture The predominating cell types are the young lymphocyte (*5*) and the lymphoblast (*6*) The nucleus of the young lymphocyte does not stain so intensely as the nucleus in the small or large lymphocytes and the chromatin is not so densely packed The nucleus is large and occupies most of the cell leaving only a small rim of deep blue cytoplasm The nucleus of the lymphoblast has a red purple finely granular chromatin structure and stains less intensely than any of the more mature forms Three of the lymphoblasts in this film have nucleoli The cytoplasm of the lympho

blast is not abundant and stains a deep blue similar to that of the young lymphocyte The lymphoblast (6) may be contrasted with the small (3) and large lymphocytes (4) that are present in the film Broken cells (7) or smudges are seen in increased numbers in acute leukemias and are noted but not included in the differential count of white cells

The red cells show greater variation in size and shape in the lower picture than in the upper picture and there is a marked decrease in the number of platelets a frequent finding in acute leukemia

(b) INTERPRETATION This picture may be compared with the upper picture showing the chronic form of the disease This might be either an acute leukemia or the terminal stage of a chronic form The presence of lymphoblasts changes in the red cells associated with the anemia and thrombocytopenia as shown in this film are diagnostic points that give a serious prognosis for the patient This patient lived only 3 weeks after this examination of his blood was made

Anemia in these leukemias may be the result of acute or chronic blood loss because of the thrombocytopenia or it may be a myelophthisic anemia showing nucleated red cells and myelocytes resulting from infiltration of the bone marrow

21 PLATE XV PLASMA-CELL LEUKEMIA

1 *DESCRIPTION OF UPPER PICTURE*

The predominating cells in this blood film are the mature plasma cells [190] which are characterized by an elongated or oval shape with an eccentric nucleus (5) and in some by a perinuclear clear area The cytoplasm is a characteristic gray green blue with a foamy or spongy structure sometimes containing vacuoles and showing ragged edges This cytoplasm is quite different from the clear blue cytoplasm of the lymphocyte (2) The mature plasma cell has a characteristic nucleus with wedges of chromatin that are distinctly separated in contrast to the more compact nuclear structure of the lymphocyte The cell with the double nucleus is a plasma cell in division The presence of dividing cells suggests the possibility of a neoplastic disease involving the plasma cell such as plasmacytoma (myeloma) or plasma cell leukemia The plasma cell is easily confused with the erythroblast (11), which may have a similar nucleus but has a gray blue homogeneous cytoplasm containing no vacuoles and a cell outline that is smooth and not ragged

The red cells show very little variation in size and shape However they appear in groups or in moderate rouleaux (9a) which are associated with hyperglobulinemia in many but not all cases The observation of rouleaux in a thin film is indicative of increased concentrations of fibrinogen or serum globulins

The platelets are normal in morphology but appear slightly decreased in number

2 *DESCRIPTION OF LOWER PICTURE*

The types of plasma cells in this picture are more varied than in the upper picture Since many stages of plasma cells are represented, these cells are more difficult to classify Two mature forms are shown (5) with characteristic eccentric nuclei oval shape irregular outline and a purple (rather than gray green blue) reaction of the cytoplasm (5b and 5c) The purple color of these cells appears to arise from a mixture of color resulting from acidophilic material and basophilic material In one of these cells (5c) acidophilic material appears as globules called Russell bodies [190 230]

The young plasma cells (6) have a gray green blue cytoplasm with some vacuolated areas but the nuclear structure is finer than that of the mature cells These cells are exceedingly difficult to distinguish from the large lymphocyte (3) in which the cytoplasm is a clear blue The characteristic gray green blue or muddy blue of the immature plasmacyte aids in its identification Also these cells are usually seen in association with some mature forms

There are two blast forms present one is the youngest form of the plasmacytic series (7) and has the characteristic blue cytoplasm and vacuoles The other blast (8) may be considered an unidentified blast form or a stem cell The stem

cell is usually much larger than the other blasts and has basophilic cytoplasm staining a much deeper blue than that of blast forms The nucleus is finely granular, stains less intensely than the cytoplasm and contains several nucleoli which are large and distinct Stem cells may be seen in any leukemic blood or severe infection that demands young cells from the hematopoietic tissues Sometimes a leukemia characterized by young cells of this type is called a stem cell leukemia

This film also shows a normal monocyte (*4*) and a normoblast (*10*) The red cells show the rouleau formation that is characteristic of the diseases with high globulin concentrations

3 *INTERPRETATION OF BOTH PICTURES*

(*a*) Peripheral Blood These pictures represent two cases of plasma cell leukemia which is a rare condition [203 227] Diagnosis is not difficult when the cells are typical and mature as in the upper picture The problem is much greater in the lower picture where many stages of plasma cell development are seen By carefully studying these cells the same characteristic cytoplasm (gray green blue vacuolated or spongy) can be seen Plasma cell leukemia is considered as the leukemic phase of plasmocytoma (multiple myeloma) [146] In the blood of both patients shown in Plate XV there was a myelophthisic anemia

(*b*) Bone Marrow Studies in Plasmocytoma A study of bone marrow aspirations in multiple myeloma may give varied pictures in different cases In some instances the mature plasma cell such as is seen in the upper picture predominates while in others the young plasma cell or the plasmablast may predominate These young forms of plasma cells have the same characteristics as the so called myeloma cell [18 19 74 201] In some cases the bone marrow has the variety of cells shown in the lower picture of plasma cell leukemia

(*c*) Plasma Cells in Other Conditions Cells of the plasmacytic series may appear in the bone marrow and peripheral blood in instances of hyperplasia of the hematopoietic system and in many infections such as measles infectious mononucleosis chicken pox [132] overwhelming sepsis and serum sickness [158] and in the recovery phase of agranulocytosis [54 170]

The Turk irritation form reported by many observers in infectious diseases is probably a cell of the plasmacytic series

(*d*) Histochemistry The periodic acid Schiff method of McManus and Hotchkiss has been used together with other techniques to investigate the cytochemistry of plasma cells [230] Evidence is presented to show that the cytoplasm of human plasma cells contains mucoprotein [230] which is a polysaccharide containing globulin If the function of the plasma cell is to produce mucoproteins then the formation of Russell bodies may represent a disorder due to overstimulation or overproduction The presence of mucoprotein in the cytoplasm and Russell bodies may account for their similarity in staining as shown by the acidophilic cytoplasm and the Russell bodies in cells *5b* and *5c*

(*e*) Anemia Anemia is a frequent complication of the infiltration of bone marrow by plasma cells and is usually myelophthisic

22 PLATE XVI INFECTIOUS MONONUCLEOSIS

1 *DESCRIPTION OF UPPER PICTURE* The first impression when one looks at a blood film like that shown in the upper picture is that of a lymphocytosis On more careful study it becomes a question whether these cells are lymphocytes or monocytes for they have many abnormal features The predominating cell is an abnormal or atypical lymphocyte (*4*) showing the following characteristics large size being as large as or larger than the monocyte (*3*) shown in the same field irregular shape of the cytoplasm irregular shape of the nucleus and clear blue cytoplasm which varies in intensity of staining The periphery appears deeper blue than other areas as if the cytoplasm were compressed There may be red or purple granules present in the cytoplasm but these are scattered and not peppered through the cytoplasm as in the normal monocyte (*3*) In the atypical lymphocytes the chromatin of the nucleus is not so condensed as

in the small lymphocyte but there may be aggregations of chromatin around the edge or in clumps through the nucleus The chromatin is less condensed than in the small lymphocyte (2) but is not as lacy or reticular in structure as in the monocyte The atypical lymphocytes apparently are more fragile than normal white cells and may appear as fragmented forms in fixed preparations The group of atypical lymphocytes in the upper drawing corresponds to the Type II described by Downey [82] and to those illustrated in the report of Baldridge Rohner and Hansmann [11]

The red cells and platelets in this film are normal

2 *DESCRIPTION OF LOWER PICTURE* The blood film represented here is a real problem in diagnosis because practically all the white cells are abnormal A careful study shows that there is one normal small lymphocyte (2) and a blast form (7) with fine granular chromatin structure of the nucleus and deep blue cytoplasm The fact that most of the cells are immature as indicated by their deep-blue cytoplasm makes classification more difficult From the characteristics of these cells alone differentiation between the monocytic series and lymphocytic series cannot be made with certainty In fact these cells are similar to some histiocytes and may be histiocytes from lymphatic tissue. However the occurrence of immature forms (6) together with the cells containing vacuolated cytoplasm (5) suggests hyperplasia of lymphoid tissue due to infectious mononucleosis These abnormal young forms, which usually are called immature atypical lymphocytes are similar to those described by Downey as Type III and the vacuolated forms correspond to Downey s Type I [82]

Plasma cells (8) are also common when there is a lymphoid hyperplasia. The plasma cell shown here is also atypical in that the nucleus is not eccentric

The platelets appear to be normal and the red cells show slight variation in size

3 *INTERPRETATION OF BOTH PICTURES*

(a) Morphology of White Cells in Infectious Mononucleosis The blood pictures represented here are from two different patients with infectious mononucleosis These changes are also seen in different stages of the disease in the same patient It is difficult, however to correlate the degree of immaturity of the white cells seen in the blood film with the severity of the illness of the patient or with the phase of disease The pictures are characterized by pathologic mononuclear cells that do not conform to normal stages in development of either the monocytic or the lymphocytic series The study of these cells by means of supravital technic [102, 166 304] has led to the conclusion that they belong to the lymphocytic series Some of the immature forms have nucleoli but the nucleus is more irregular in shape and the chromatin is more clumped than in the typical lymphoblasts The cells in this plate should be carefully compared with those of Plates XII, XIII XIV, and XV to appreciate the abnormal features of these cells

The review by Bernstein [22] gives many important facts about the course of the disease These abnormal cells may exist early in the disease but typically they appear within the first 4 or 5 days of illness and rapidly reach a maximum within a week or 10 days The proportion of mononuclear atypical lymphocytes in the cases studied varied from 40 to 90 percent Some of these abnormal forms may persist for 9 or 10 months after the patient has recovered from clinical signs of the disease

(b) White Cell Count The white-cell count may show a marked leukopenia during the first week but a leukocytosis characterized by atypical and normal lymphocytes is the rule In 65 cases studied Bernstein found that 86 percent of the white cell counts were between 8 0 and 20 0 × $10^3/mm^3$ [22] In a few instances the white cell counts exceeded 40 0 × $10^3/mm^3$

(c) Forms of White Cells Other than Atypical Lymphocytes The changes in the blood picture should be followed during the course of the disease as in all infections The number of eosinophils is decreased during the acute phase as in many infections but increases during convalescence Myelocytes may be present in patients who are severely ill The neutrophils are characterized by immature forms (bands) often with toxic granulation

(d) Red Cells and Platelets. It is important

to emphasize that the red cells in uncomplicated infectious mononucleosis are normal This is in contrast to the frequent occurrence of anemia as a complication of leukemia However the blood picture of infectious mononucleosis may be superimposed on that of any other condition including diseases involving red cells such as hypochromic anemia hemolytic jaundice anemia due to hemorrhage or dietary deficiency Stippled cells have been seen in some cases

The platelets are usually normal in infectious mononucleosis However a few cases with thrombocytopenia have been reported [22]

(*e*) Heterophile Antibody The Paul Bunnell test for heterophile antibody is positive in most cases of infectious mononucleosis at some time during the course of the disease The heterophile antibody in this disease is not absorbed by guinea pig kidney a test that is highly specific for infectious mononucleosis [116 228]

(*f*) General Features of Infectious Mononucleosis Infectious mononucleosis may be associated with abnormalities of almost any system or organ thus giving protean manifestations The outstanding features of glandular fever are fever lymphadenopathy sore throat malaise headache and gastro intestinal symptoms There is usually a mild or moderate degree of hepatitis

(*g*) Differentiation from Leukemia Infectious mononucleosis is often diagnosed as leukemia because of the bizarre forms and immaturity of the white blood cells In the leukemias however anemia and thrombocytopenia usually are evident by the time the patient seeks medical attention The heterophile test is uniformly negative in leukemia In infectious mononucleosis anemia and thrombocytopenia are not characteristic features of the disease The differentiation of the cell types has been discussed here and in the description of Plate XIV

(*h*) Atypical Lymphocytes in Conditions Other than Infectious Mononucleosis Atypical lymphocytes similar to the varieties seen in infectious mononucleosis are found in a number of other conditions In some cases the clinical features of the illness are sufficient to make a differential diagnosis in some the use of the heterophile test with differential absorption of the antibodies is necessary to differentiate infectious mononucleosis from conditions giving a similar blood picture Young lymphocytes and all stages of plasma cells may be seen in increased numbers in many of these conditions in which the atypical lymphocytes appear

Infectious hepatitis An excellent description of the abnormal lymphocytes in infectious (epidemic) jaundice is reported by Minot and Jones [196] and has been confirmed by more recent studies [12 122] Although the white cell count is seldom above normal and often shows leukopenia [286] there is a relative lymphocytosis which frequently shows atypical lymphocytes young lymphocytes and cells of the plasmacytic series The heterophile test in infectious (epidemic) hepatitis is uniformly negative This is of importance because most patients with infectious mononucleosis have hepatitis which may not be demonstrable clinically but is occasionally associated with jaundice

Serum sickness The abnormal lymphocytes in serum sickness may be indistinguishable from those of infectious mononucleosis The atypical forms include irregularly shaped vacuolated and immature lymphocytes which may constitute as many as 30 percent of the white cells Plasma cells of all stages also appear in increased numbers The unabsorbed serum may show a high concentration of heterophile antibody However the heterophile antibody (Forssman antibody) of serum sickness is completely absorbed by guinea pig kidney which does not remove the heterophile antibody of infectious mononucleosis [116]

Chicken pox In an excellent study of the blood picture in 56 patients with varicella Holbrook [132] has shown that there is an increase of lymphocytes during the leukopenic phase of the disease During this stage there is an increase of young lymphocytes lymphoblasts and abnormal forms some showing mitotic figures An increase in plasma cells also occurred in 50 percent of the cases to levels as high as 6 or 8 percent in some instances In another study [57] a case has been observed with the following percentages of cells in the differential count lymphocytes 27 young lymphocytes 17 irregular lymphocytes 5 vacuolated lymphocytes 2 and in addition cells of the plasmacytic series 7

Primary atypical pneumonia A study of the blood picture in 28 cases of virus pneumonia including 126 white cell counts, showed that 68 counts (54 percent) were within the normal range (5 to 10 × 10³/mm³) 5 counts were under 5 × 10³/mm³ and 53 counts were over 10 × 10³/mm³ [57] Many of these higher counts occurred during the third and fourth weeks of the disease A greater number of lymphocytes is found early in the disease than is seen in pneumococcus pneumonia There was a slight to moderate increase of young and irregular lymphocytes such as is seen in infectious mononucleosis during this lymphoid response One patient showed up to 9 percent and another 5 percent of these cells Plasma cells were occasionally seen

Other conditions Abnormal lymphocytes may be present in normal individuals especially children in hyperthyroidism typhoid fever and infections other than the virus diseases These observations suggest that the appearance of atypical lymphocytes is of no single diagnostic significance but apparently indicates a particular type of response of lymphocytic tissue

(*i*) Lymphocytosis and Leukemoid Retions of Lymphocytic Type Lymphocyto either absolute or relative, is seen in many vir infections at some stage in the disease and i other infectious diseases during the period of re covery The atypical cells characteristic of infec tious mononucleosis are not usually present

Relative lymphocytosis is the rule in conditions associated with neutropenia, for example pernicious anemia aplastic anemia, agranulocytosis overwhelming sepsis influenza typhoid fever typhus fever malaria measles and mumps

Some of the conditions showing an absolute lymphocytosis are acute infectious lymphocytosis of unknown etiology [8, 270] thyrotoxicosis [57 127 188] German measles (rubella) [178] whooping cough typhoid fever and congenital syphilis [11] Lymphocytosis simulating lymphc cytic leukemia has been reported in two cases o miliary tuberculosis [104], in a case of adenocar cinoma of the sigmoid [238] and in a patient with carcinoma of the breast with metastasis to lungs and spleen [156] (A49 60 Mallory Insti tute of Pathology)

REFERENCES

1 Abt A F Mononuclear erythrophagocytos s n the blood of a newborn nfant *Am J D s Ch ld* 42 1364–1371 (1931)

? Abt A F and Bloom W Essent al l po d h st o cytos s (type N emann P ck) A congen tal d s turbance in l po d metabol sm n nfants a soc ate l w th a plenohepatomegaly *J A M A* 90 2076–2080 (1928)

3 Ackerman G A M croscop c and h tochem cal stud es on the Auer bod es n leukem c cells *Blood* 5 847 863 (1950)

4 Aksov M Lehmann H and L e Injo L E The recogn t on of haemoglob ns A_2 and F *Lancet* 1 792 793 (1957)

5 Alder A Ueber morpholog sche Veranderungen an len we ssen Blutkorperchen be Infekt ons krankhe ten *Schwe med W cl s h* 19 440–442 (1921)

6 Al son A C Notat on for hemoglob n types and genes controll ng the r syndromes *Sc e e* 122 640 (1955)

7 A mand Del le P F Hurst A R and Sorapure V E Fam l al eos noph l a *Guy s Hosp Rep* 80 248–252 (1930)

8 Arne l G C and R ley I D Acute nfect ous lymphocytos s *Qua t J Med* (n s) 21 285–290 (1952)

9 Arneth J D neutroph len Leukozy ten be Infek t onskrankhe ten *Deutsc l e med W cl n chr* 30 54–56 (1904)

10 Auer J Some h therto undescr bed structures n the large lymphocy tes of a ca e of acute leukem a *Am J M Sc* 131 100? 1015 (1906)

11 Baldr dge C W Rohner F J and Hansmann G H Glandular fever (nfect ous mononucleo s s) *Arch Int Med* 38 413–448 (19?6)

12 Ba ker H M Capps R B and Allen F W Acute nfect ous hepat s n the Med erranean theater *J A M* 1 128 997 1003 (1945)

13 Ba nett C W Unavo dable erro n d fferent al count of leukocytes of blood *J Cl In e g t on* 12 7 85 (1933)

14 Ba ret A M A spec al form of ery th ocyte pos es ng ncreased r s stance to hypoton c sal ne *J Pa h & Ba t* 46 603–618 (1938)

15 Ba sen F A and Kornzw g A L Malforma t on of the erythrocyt s n a case of atyp cal re n t s p gmentosa *Blood* 5 381 387 (1950)

16 Battle J D Jr and Lew s L A S ckle cell tra t and thalassem a m nor *Cl n Re ea l Proc* 1 3 (1953)

17 Baty J M L po d h st ocytos (N emann s d sease) *Am J D Ch ld* 39 573 5 5 (1930)

18 Bayrd E D The bone marro v on sternal asp ra t on n mult ple myeloma *Blood* 3 98 1018 (1948)

19 Bayrd E D and Heck F J Mult ple myeloma A rev ew of e ghty three pro ed cases *J A M* 1 133 147 157 (1947)

20 Begemann N H and Campagne A V Homo zygous form of Pelger Huet nuclear anomaly n man *Acta haemat* 7 295–303 (195?)

21 Beld ng H W Daland G A and Parker F Jr H st ocyt c and monocyt c leukemia a cl n cal hematolog cal and patholog cal d fferent at on *Cancer* 8 237 25? (1955)

22 Be nstein A Infect ous mononucleos s *Med ne* 19 85 159 1940

23 Blackfan K D and D amond L K The monocyte n act ve tuberculos s Suprav tal stud es of the blood *Am J D s Cl ld* 37 233 243 (1929)

24 Blackfan K D D amond L K and Le ster C M *A las of the blood h ldren* (The Common wealth Fund Ne v York 1944) Plate 49

25 Block M and Jacobson L O Myelo d m ta plas a *J A M A* 143 1390–1396 (1950)

26 Bloom W The h stogenes s of essent al l po d h stocytos s (N emann P ck s disease) *Arch Path* 6 827 859 (1928)

27 Bo os J von D e Behandlung der Anäm en *E geb d in Med u Kinde h* 42 635–740 (193?)

28 Bover R M Howell Jolly bod es and splen c at ophy *Guy s Hosp Rep* 91 81 90 (1942)

29 Bradford W L and Dye J Obse vat ons on the morphology of the erythrocytes n Med ter ranean disease — thalas emia (erythroblast c anem a of Cooley) *J Ped at* 9 312 317 (1936)

30 Breche G and Cronk te E P Morphology and enumerat on of human blood p atelets *J Appl ed Ph y ol* 3 365–377 (1950)

31 Brew ter H H and Wol enman O J Myelo d metaplas a of the spleen w th acute hemo yt c anem a *New England J Med* 227 822 825 (1942)

32 Broustet P Sar c Leger Bergaud A and Bor chard Mle Cellule endothel o des hémoh st o b ast ques et var at on de la formule du sang cap l a re et du sang veineux dans la malad e d O ler *Ar l d mal d c coeur* 39 294–300 (1946)

33 Buckman T E Splenomegaly in nfants and ch dren *M Cl n No th Ame a* 8 1551 1575 (1925)

34 Bunt ng C H Ce l react ons n res tance and mmun ty *W ons n M J* 24 305–308 (1925)

35 Cabot R C R ng bod es (nuclear remnants) n anem c blood *J M Re ear h* 4 15–18 (1903)

36 Cam nopetros J Recherches sur l anem e eryth roblast que nfant le d peup e de la Méd terranée or enta e *Ann de méd* 43 104–125 (1938)

37 Carpenter G and Flory C M Chron c non leukem c myelos s report of a case w tl mega karyocyt c myelo d splenomegaly leukoerythroblas

tic anemia generalized osteosclerosis and myelofibrosis *Arch Int Med* 67 489–508 (1941)

38 Casey A E Nettles T E and Hidden E H Basophilic leukemia *South M J* 39, 325–332 (1946)

39 Castle W B Disorders of the blood Chap 28 in Sodeman W A *Pathologic physiology Mechanisms of disease* (W B Saunders Philadelphia 1956)

40 Castle W B and Daland G A 'Susceptibility of mammalian erythrocytes to hemolysis with hypotonic solutions *Arch Int Med* 60 949–966 (1937)

41 Chernoff A I The human hemoglobins in health and disease *New England J Med* 253, 322–331 365–374 416–423 (1955)

42 Chernoff A I Minnich V and Chongcharoensuk S Hemoglobin E a hereditary abnormality of human hemoglobin *Science* 120 605–606 (1954)

43 Chernoff A I Minnich V Chongcharoensuk S Na Nakorn S and Chernoff R Clinical hematological and genetic studies of hemoglobin E *J Lab & Clin Med* 44 780–781 (1954)

44 Chernoff A I and Singer K Studies on abnormal hemoglobins IV Persistance of fetal hemoglobin in the erythrocytes of normal children *Pediat* 9 469–474 (1952)

45 Choremis C and Zannos L Microdrepanocytic disease in Greece *Blood* 12 454–460 (1957)

46 Claudon D B and Holbrook A A Fatal aplastic anemia associated with chloramphenicol (chloromycetin) therapy report of 2 cases *J A M A* 149 912–914 (1952)

47 Clough P W Monocytic leukemia ' *Bull Johns Hopkins Hosp* 51 148–177 (1932)

48 Committee for clarification of the nomenclature of cells and diseases of the blood and blood forming organs condensation of first two reports *Blood* 4 89–96 (1949)

49 Conn H J *Biological stains a handbook on the nature and use of the dyes employed in the biological laboratory* (Biotech Publications Geneva N Y ed 5 1946)

50 Conn H O Sickle-cell trait and splenic infarction associated with high altitude flying *New England J Med* 251 417–420 (1954)

51 Cooley T B and Lee P The rôle of erythrocyte fragmentation in the genesis of anemia *J Pediat* 3 55–63 1933

52 Coventry W D Cyclic neutropenia report of a case treated by splenectomy *J A M A* 153 28–31 (1925)

53 Cunningham R S Sabin F R and Doan C A *The development of leucocytes lymphocytes and monocytes from a specific stem cell in adult tissues* Carnegie Institution of Washington Pub No 361 (1925) p 227 (Contributions to embryology No 84)

54 Custer R P *Atlas of blood and bone marrow* (W B Saunders Philadelphia 1949)

55 Dacie J V *The haemolytic anaemias congenital and acquired* (Grune & Stratton New York 1954)

56 Dacie J V and White J D "Erythropoiesis with particular reference to its study by biopsy of human bone marrow a review" *J Clin Path* 2 1–32 (1949)

57 Daland G A personal observations

58 Daland G A and Castle W B, A simple and rapid method for demonstrating sickling of the red blood cells the use of reducing agents *J Lab & Clin Med* 33 1082–1088 (1948)

59 Daland G A Gottlieb L Wallerstein R O and Castle W B Hematologic observations in bacterial endocarditis especially the prevalence of histiocytes and the elevation and the variation of the white cell count in blood from the ear lobe *J Lab & Clin Med* 48 827–845 (1956)

60 Daland G A and Hanson M L unpublished data

61 Daland G A Heath C W and Minot G R Differentiation of pernicious anemia and certain other macrocytic anemias by the distribution of red blood cell diameters *Blood* 1 67–75 (1946)

62 Daland G A and Strauss M B The genetic relation and clinical differentiation of Cooley s anemia and Cooley s trait *Blood* 3 438–448 (1948)

63 Dameshek W Acute monocytic (histiocytic) leukemia review of the literature and case reports *Arch Int Med* 46 718–740 (1930)

64 Dameshek W Proliferative diseases of the reticulo endothelial system aleukemic reticulosis *Folia haemat* 49 64–85 (1933)

65 Dameshek W ' Target cell anemia anerythroblastic type of Cooley s erythroblastic anemia *Am J M Sc* 200 445–454 (1940)

66 Dameshek W Familial Mediterranean target oval cell syndromes *Am J M Sc* 205 643–660 (1943)

67 Dameshek W Henstell H H and Valentine E H The comparative value and the limitations of the trephine and puncture method for biopsy of the sternal bone marrow" *Ann Int Med* 11 801–818 (1937)

68 Dameshek W, and Ingall M Agranulocytosis (malignant neutropenia) Report of nine cases two with recovery *Am J M Sc* 181 502–512 (1931)

69 Dameshek W and Miller E B The megakaryocytes in idiopathic thrombocytopenic purpura a form of hypersplenism *Blood* 1 27–51 (1946)

70 Dausset J Agranulocytoses et leucopenies immunologiques aspects serologiques et serologie des leucocytes en general *Sang* 25 683–706 (1954)

71 Dawbarn R Y Earlam F and Evans W H The relation of the blood platelets to thrombosis after operation and parturition *J Path & Bact* 31 833–873 (1928)

72 Degos R, Ossipowski B and Baranger H Étude Critique des Leucémies a Monocytes *La Presse Médicale* 70 1441–1445 (1954)

73 Diggs L W and Bibb J The erythrocyte in sickle cell anemia morphology size hemoglobin content fragility and sedimentation rate *J A M A* 112 695–700 (1939)

74 Diggs L W and Sturgeon M S A study of the sternal marrow and peripheral blood of fifty five patients with plasma cell myeloma, *J Lab & Clin Med* 32 167–177 (1947)

75 Doan C A Cunningham R S and Sabin F R *Experimental studies on the origin and maturation of avian and mammalian red blood cells* Carnegie Institution of Washington Pub No 361 (1925) pp 163–226 (Contributions to embryology)

76 Doan C A and Reinhart H L The basophil granulocyte basophilcytosis and myeloid leukemia basophil and mixed granule types an experimental, clinical and pathological study with the report of a new syndrome *Am J Clin Path* 11 1–40 (1941)

77 Doan C A and Sabin F R Normal and pathological fragmentation of red blood cells the phagocytosis of these fragments by desquamated endothelial cells of the blood stream the correlation of the peroxidase reaction with phagocytosis in mononuclear cells *J Exper Med* 43 839 850 (1926)

78 Doniach I Gruneberg H and Pearson J E G, The occurrence of siderocytes in adult human blood *J Path & Bact* 55 23–31 (1943)

79 Downey H Monocytic leucemia and leucemic reticulo-endotheliosis in Downey H *Handbook of hematology* (Paul B Hoeber Inc New York Vol. 2 1938) pp 1275–1333

80 Downey H Diseases of the blood in Bell E T *A textbook of pathology* (Lea & Febiger Philadelphia 1934) p 675

81 Downey H Major S G and Noble J F Leukemoid blood pictures of the myeloid type *Folia haemat* 41 493–511 (1930)

82 Downey H and McKinlay C A Acute lymphadenosis compared with acute lymphatic leukemia *Arch Int Med* 32 82–112 (1923)

83 Duke W W Causes of variation in the platelet count *Arch Int Med* 11 100–120 (1913)

84 Ehrlich, P Über schwere anämische Zustande *Verhandl d Kong f inn Med* 11 33–64 1892

85 Ehrlich P *Farbenanalytische Untersuchungen zur Histologie und Klinik des Blutes* (Hirschwald Berlin 1891)

86 Ehrlich P and Lazarus A *Anaemia* (Rebman Co New York Eng ed 1910) p 68

87 Emerson C P Ham T H and Castle W B Hemolytic action of certain organic oxidants derived from sulfanilamide phenylhydrazine and hydroquinone *J Clin Investigation* 20 451 (1941)

88 Emmel V E A study of the erythrocytes in a case of severe anemia with elongated and sickle shaped red blood corpuscles *Arch Int Med* 20 586–598 (1917)

89 Epstein R D Cells of the megakaryocyte series in pernicious anemia in particular the effect of specific therapy *Am J Path* 25 239–251 (1949)

90 Evans F A Observations on the origin and status of the so called transitional white blood cell *Arch Int Med* 17 1–12 (1916)

91 Evans T S Monocytic leukemia (general review of subject) *Medicine* 21 421–456 (1942)

92 Falconer E H The clinical significance of punctate basophilia in the erythrocyte *Ann Int Med* 12 1429–1441 (1939)

93 Ferrata A and Franco E Cellule istiodi (emoistioblasti) e loro derivati nel sangue circolante *Arch per le sc med* 42 109–122 (1919)

94 Ferrata A. and Storti E. *Le malattie del sangue manuale per medici e studenti* (Societe Editrice Libraria Milan 1946)

95 Fertman M H and Doan C A Irreversible toxic inclusion body anemia A rarely recognized syndrome clinical and experimental studies *Blood* 3 349–360 (1948)

96 Fertman M H and Fertman M B Toxic anemias and Heinz bodies *Medicine* 34 131–19? (1955)

97 Foord A G Parsons L and Butt E M Leukemic reticulo-endotheliosis (monocytic leukemia) with report of cases *J A M A* 101 1859–1866 (1933)

98 Foot N C The endothelial phagocyte A critical review *Anat Rec* 30 15–51 (1925)

99 Forkner C E Clinical and pathologic differentiation of the acute leukemias with special reference to acute monocytic leukemia *Arch Int Med.* 53 1–34 (1934)

100 Frerichs J B The influence of a monocytosis of the peripheral blood stream upon the cellular character of an acute inflammation *Bull Johns Hopkins Hosp* 74 49–54 1944

101 Fuente V de la Megakaryocytes in normal and in thrombocytopenic individuals *Blood* 4 614–630 (1949)

102 Gall, E A. Diagnostic value of supravital staining in infectious mononucleosis *Am J M Sc* 194 546–554 (1937)

103 Gall E A and Mallory T B Malignant lymphoma a clinico-pathologic survey of 618 cases *Am J Path 18 381–450 (1942)*

104 Gardner F H and Mettier S R Lymphocytic leukemoid reaction of the blood associated with miliary tuberculosis *Blood* 4 767–775 (1949)

105 Giffin H Z and Holloway J K A review of twenty-eight cases of purpura hemorrhagica in which splenectomy was performed *Am J M Sc* 170 186–206 (1925)

106 Goldner F M and Mann W N The statistical error of the differential white count *Guy's Hosp Rep* 88 54–65 (1938)

107 Goodwin A F Some new observations on Auer bodies in acute myelogenous leukemia *Folia haemat* 51 359–366 (1934)

108 Gordin R Toxic granulation in leukocytes development and relation to cloudy swelling *Acta med Scandinav* 143 Supplement 270 (1952)

109 Greenberg M S Kass E H and Castle W B Studies on the destruction of red blood cells XII Factors influencing the role of S hemoglobin in the pathologic physiology of sickle cell anemia and related disorders *J Clin Investigation* 36 833–843 (1957)

110 *Griggs R C and Harris J W The biophysics of the variants of sickle-cell disease A M A Arch Int Med* 97 315–326 (1956)

111 Gruneberg H The anemia of flexed tailed mice II Siderocytes *J Genetics* 44 246–271 (1942)

112 Gruneberg H Siderocytes a new kind of erythrocyte *Nature* 148 114–115 (1941)

113 Haden R L The mechanism of the increased fragility of the erythrocytes in congenital hemolytic jaundice *Am J M Sc* 188 441–461 (1934)

114 Hahn E V and Gillespie E B Sickle cell anemia report of a case greatly improved by splenectomy *Arch Int Med* 39 233–254 (1927)

115 Hall B E and Watkins C H Myelogenous leukemia changing to monocytic leukemia *Am J Clin Path* 11 443–459 (1941)

116 Ham T H *A syllabus of laboratory examinations in clinical diagnosis* (Harvard University Press Cambridge Mass 1950)

117 Ham T H Castle W B Gardner F H and *Daland G A Laboratory diagnosis of polycythemia* and anemia *New England J Med* 243 815–827 860–872 (1950)

118 Ham T H Shen S C Fleming E M and Castle W B Studies on the destruction of red blood cells IV Thermal injury Action of heat in causing increased spheroidicity osmotic and mechanical fragilities and hemolysis of erythrocytes observations on the mechanisms of destruction of such erythrocytes in dogs and in a patient with a fatal thermal burn *Blood* 3 373–403 (1948)

119 Ham T H Wiseman R Jr and Hinz C F Jr Mechanisms of destruction of red cells in certain hemolytic conditions *A M A Arch Int Med* 96 574–597 (1956)

120 Harris J W Brewster H H Ham T H and Castle W B Studies on the destruction of red blood cells X The biophysics and biology of sickle-cell disease *A M A Arch Int Med* 97 145–168 (1956)

121 Hartz W H Jr and Schwartz S O Hemoglobin C disease Report of 4 cases *Blood* 10 235–246 (1955)

122 Havens W P and Marck R E The leukocyte response of patients with experimentally induced infectious hepatitis *Am J M Sc* 212 129–138 (1946)

123 Heath C W and Daland G A The life of reticulocytes experiments on their maturation *Arch Int Med* 46 533–551 (1930)

124 Heck F J and Hall B E Leukemoid reactions of myeloid type *J A M A* 112 95–101 (1939)

125 Heinz R Morphologische Veranderungen der rothen Blutkorperchen durch Gifte *Virchow Arch f path Anat* 122 112–116 (1890)

126 Hematology Department U S Naval Medical School *Hematology* (U S Naval Medical School National Naval Medical Center Bethesda Maryland 1945)

127 *Hertz S and Lerman J The blood* picture in exophthalmic goitre and its changes resulting from iodine and operation — a study by means of the supravital technique *J Clin Investigation* 11 1179–1196 (1932)

128 Hickling R A Chronic non leukaemic myelosis *Quart J Med* 6 253–275 (1937)

129 Hill J M and Duncan C N Leukemoid reactions *Am J M Sc* 201 847–857 (1941)

130 Hill L W Report on leucocyte inclusion bodies *Boston M & S J* 170 792–794 (1914)

131 Hills A G Forsham P H and Finch C A Changes in circulating leukocytes induced by the administration of pituitary adrenocorticotropic hormone (ACTH) in man *Blood* 3 755–768 (1948)

132 Holbook A A The blood picture in chicken pox, *Arch Int Med* 68 294–308 (1941)

133 Huet G J Über eine bisher undekannte familiare Anomalie de Leukocyten *Klin Wchnschr* 11 *1264–1266 (1932)*

134 Humble J G Anderson I White J C and Freeman T A family illustrating the double inheritance of the sickle cell trait and of Mediterranean anaemia *J Clin Path* 7 201–208 1954

135 Hunter F T Drug or protein allergy as a cause of agranulocytosis and certain types of purpura *New England J Med 213 663–674 (1935)*

136 Isaacs R The refractive granule red blood corpuscle its behavior and significance *Anat Rec* 29 299–313 (1925)

137 Isaacs R Brock B and Minot G R The resistance of immature erythrocytes to heat *J Clin Investigation* 1 425–433 (1925)

138 Israels M C G The reticuloses a clinicopathological study *Lancet* 2 525–530 (1953)

139 Israels M C G *An atlas of bone marrow pathology* (Grune & Stratton New York 1948)

140 Itano H A Clinical states associated with alterations of the hemoglobin molecule *A M A Arch Int Med* 96 287–297 (1955)

141 Itano H A and Pauling L A rapid diagnostic test for sickle cell anemia *Blood* 4 66–68 (1949)

142 Jackson H Jr The protean character of the leukemias and of the leukemoid states *New England J Med* 220 175–181 (1939)

143 Jackson H Jr and Merrill D Agranulocytosis

angina associated with the menstrual cycle *New England J Med* 210 175–176 (1934)

144 Jackson H Jr and Parker F Jr Agranulocytosis its etiology and treatment *New England J Med* 212 137–148 (1935)

145 Jackson H Jr and Parker F Jr *Hodgkin s disease and allied disorders* (Oxford University Press New York 1947)

146 Jackson H Jr Parker F Jr and Bethea J M Studies of diseases of the lymphoid and myeloid tissues II Plasmatocytoma and their relation to multiple myelomata *Am J M Sc* 181 169–180 (1931)

147 Jackson H Jr Parker F Jr and Lemon H M Agnogenic myeloid metaplasia of the spleen a syndrome simulating other more definite hematologic disorders *New England J Med* 222 985–994 (1940)

148 Jaffee R H Aleukemic myelosis *Arch Path & Lab Med* 3 56–72 (1927)

149 Jarcho S Diffusely infiltrative carcinoma a hitherto undescribed correlation of several varieties of tumor metastasis *Arch Path* 22 674–696 (1936)

150 Jones O in H Downey ed *Handbook of hematology* (Hoeber New York 1938) p 2101

151 Jordan W S Prouty R L Heinle R W and Dingle J H The mechanism of hemolysis in paroxysmal cold hemoglobinuria *Blood* 7 387–403 (1942)

152 Jordans G H W Hereditary granulation anomaly of the leucocytes (Alder) *Acta med Scandinav* 129 348–351 (1948)

153 Kaplan E Zuelzer W W and Neel J V A new inherited abnormality of hemoglobin and its interaction with sickle cell hemoglobin *Blood* 6 1240–1259 (1951)

154 Kaplan E Zuelzer W W and Neel J V Further studies on hemoglobin C II The hematologic effects of hemoglobin C alone and in combination with sickle cell hemoglobin *Blood* 8 735–746 (1953)

155 Kennedy P C and Finland M Fatal agranulocytosis from sulfathiazole *J A M A* 116 295–296 (1941)

156 Kleeman C R Lymphocytic leukemoid reaction associated with primary carcinoma of the breast *Am J Med* 10 522–527 (1951)

157 Klein A Hussar A E and Bornstein S Pelger Huet anomaly of the leukocytes *New England J Med* 253 1057–1062 (1955)

158 Kolouch F Good R A and Campbell B The reticulo-endothelial origin of the bone marrow plasma cells in hypersensitive states *J Lab & Clin Med* 32 749–755 (1947)

159 Korst D R Clatanoff D V and Schilling R F On myelofibrosis *A M A Arch Int Med* 97 169–183 (1956)

160 Krumbhaar E B Leukemoid blood pictures in various clinical conditions *Am J M Sc* 172 519–533 (1926)

161 Krumbhaar E B The changes produced in the blood picture by removal of the normal mammalian spleen *Am J M Sc* 184 215–228 (1932)

162 Kugel M A and Rosenthal N Pathological changes in polymorphonuclear leukocytes during progress of infections *Am J M Sc* 183 657–667 (1932)

163 Kunkel H G and Wallenius G New hemoglobin in normal adult blood *Science* 122 288 (1955)

164 Lawrence J S Leukopenia its classification etiology and treatment *Tr and Stud Coll Physicians Philadelphia* 14 1–14 (1946)

165 Lawrence J S Neel J V and Itano H Hemolytic anemia presumably due to coexistence of the gene for thalassemia and sickling *Tr A Am Physicians* 65 203 213 (1952)

166 Lawrence J S and Todd H Variations in the characteristics of lymphocytes in supravital preparations *Folia haemat* 44 318–331 (1931)

167 Lee R I and Minot G R The significance of blood platelets *Cleveland M J* 16 65–88 (1917)

168 Lee R I Vincent B and Robertson O H Immediate results of splenectomy in pernicious anemia *J A M A* 65 216–220 (1915)

169 Lehman H Variations of haemoglobin synthesis in man *Acta Genetica et Statistica Medica* 6 413–429 (1956–1957)

170 Leitner S J *Bone marrow biopsy Haemotology in the light of sternal puncture* (trans and rev by C J C Britton and E Neumark Grune and Stratton New York 1949)

171 Lesses M F and Gargill S L Thiouracil as a cause of neutropenia and agranulocytosis *New England J Med* 233 803–811 (1945)

172 Lichtenstein L Histiocytosis X Integration of eosinophilic granuloma of bone Letterer Siwe Disease and Schuller Christian disease as related manifestations of a single nosologic entity *A M A Arch Path* 56 84–102 (1953)

173 Lie Injo Luan Eng Haemoglobin E in Indonesia *Nature* 176 469–470 (1955)

174 Lie Injo Luan Eng and Jo Kian Tjoy Cooley s anaemia in children in Indonesia *Docum de med geogr et trop* 7 30–42 (1955)

175 Lie Injo Luan Eng Poey Seng Hin Kho Lien Keng and Endenburg P M Chronic hypochromic microcytic anaemia associated with haemoglobin H *Acta haemat* 18 156–167 (1957)

176 Lipton E L Elliptocytosis with hemolytic anemia the effect of splenectomy *Pediat* 15 67–83 (1955)

177 Lucey H C Fortuitous factors affecting the leucocyte count in blood from the ear *J Clin Path* 3 146–151 (1950)

178 MacBryde C M and Charles C M Differential diagnosis of rubella use of the Schilling leuko-

cyte count *Arch Int Med* 56 935–943 (1935)
179 McFadzean A J S and Davis L J Iron staining erythrocytic inclusions with special reference to acquired haemolytic anaemia *Glasgow M J* 28 237–279 (1947)
180 Mackay W The blood platelet its clinical significance *Quart J Med* 24, 285–328 (1931)
181 Madison F W and Squier T L The etiology of primary granulocytopenia (agranulocytic angina) *J A M A* 102 755–759 (1934)
182 Mainland D Coady B K and Joseph S Observational variation in the differential count *Folia haemat* 54 8–21 (1936)
183 March H W Schlyen S M and Schwartz S E Mediterranean hemopathic syndromes (Cooley's anemia) in adults *Am J Med* 13 46–57 (1952)
184 Marchal G and Bargeton A. Difficultés de diagnostic entre Maladie de Hodgkin et leucémie a monocytes *Sang* 7 321–327 (1933)
185 Maximow A A and Bloom W *A textbook of histology* (W B Saunders Philadelphia ed 5 1948) p 89
186 McIntosh R and Wood C L An inquiry into the genetic factor in Cooley's anemia *Am J Dis Child* 64 192–194 (1942)
187 Medlar E M An evaluation of the leucocytic reaction in the blood as found in cases of tuberculosis *Am Rev Tuberc* 20 312–346 (1929)
188 Menkin V Relative lymphocytosis in hyperthyroidism *Arch Int Med* 42 419–424 (1928)
189 Merrill D and Jackson H Jr Renal complications of leukemia *New England J Med* 228 271–276 (1943)
190 Michels N A The plasma cell *Arch Path* 11 775–793 (1931)
191 Mickler E E and Digg I W The detection of the sickle cell trait a comparison of the sealed moist preparation using capillary blood collected during venous stasis and the sodium bisulfite method *Am J Clin Path* 20 861–864 (1950)
192 Minnich V Na Nakorn S Chongchareonsuk S and Kochaseni S Mediterranean anemia A study of thirty two cases in Thailand *Blood* 9 1–23 (1954)
193 Minot G R Megacaryocytes in the peripheral circulation *J Exper Med* 35 1–7 (1922)
194 Minot G R and Buckman T E Erythremia (Polycythemia rubra vera) The development of anemia the relation to leukemia consideration of the basal metabolism blood formation and destruction and fragility of the red cells *Am J M Sc* 166 469–489 (1923)
195 Minot G R and Buckman T E The blood platelets in the leukemias *Am J M Sc* 169 477–485 (1925)
196 Minot G R and Jones C M Infectious (catarrhal) jaundice An attempt to establish a clinical entity *Boston M & S J* 189 531–551 (1923)
197 Minot G R and Smith L W The blood in tetrachlorethane poisoning' *Arch Int Med* 28 687–702 (1921)
198 Moeschlin S Immunological agranulocytosis (clinical aspects) *Sang* 25 679–692 (1954)
199 Monasterio G and Gigli G Histioleukaemias and acute leukaemias *Acta med Scandinav* 140 381–394 (1951)
200 Montgomery H and Watkins C H Monocytic leukemia Cutaneous manifestations of the Naegeli and Schilling types hematologic differentiation *A M A Arch Int Med* 60 51–63 (1937)
201 Morissette L and Watkins C H Multiple myeloma diagnostic value of the blood smear *Proc Staff Meet Mayo Clin* 17 433–439 (1942)
202 Morris R. S Nuclear particles in the erythrocytes *Arch Int Med* 3 93–97 (1909)
203 Moss W T and Ackerman L V Plasma cell leukemia *Blood* 1 396–406 (1946)
204 Motulsky A G Genetic and haematological significance of hemoglobin H *Nature* 178 1055–1056 (1956)
205 Motulsky A G Luttgens W F Patterson W and Rotter R. Splenic infarction precipitated by airplane flight in patients with sicklemia" *Clin Research Proc* 3 51 (1955)
206 Mugrage E R and Andresen M I Value for red blood cells of average infants and children *Am J Dis Child* 51 775–791 (1936)
207 Naegeli O, *Blutkrankheiten und Blutdiagnostik* (J Springer Berlin (1931)
208 Na Nakorn S and Minnich V Studies on hemoglobin E III Homozygous hemoglobin E and variants of thalassemia and hemoglobin E A family study *Blood 12 529–538 (1957)*
209 Neel J V The inheritance of sickle cell anemia *Science* 110 64–66 (1949)
210 Neel J V The inheritance of the sickling phenomenon with particular reference to sickle cell disease *Blood* 6 389–412 (1951)
211 Neel J V Abnormal forms of hemoglobin from a genetic point of view *A M A Arch Int Med* 96 555–558 (1956)
212 Neel J V The genetics of human haemoglobin differences problems and perspectives *Ann Human Genet 21 1–30 (1956)*
213 Neel J V Human hemoglobin types their epidemiologic implications *New England J Med* 256 161–171 (1957)
214 Neel J V Itano H A and Lawrence J S Two cases of sickle cell disease presumably due to the combination of the genes for thalassemia and sickle cell hemoglobin *Blood 8 434–443* (1953)
215 Neel J V Kaplan E and Zuelzer W W Further studies on hemoglobin C I A description of three additional families segregating for hemoglobin C and sickle cell hemoglobin *Blood* 8 724–734 (1953)
216 Neel J V and Valentine W N., "The frequency of thalassemia *Am J M Sc 209 568 572* (1945)

REFERENCES

217 Olef I The differential platelet count its clinical significance *Arch Int Med* 57 1163–1185 (1936)

218 Orchard N P Letterer Siwe s syndrome report of a case with unusual peripheral blood changes *Arch Dis Childhood* 25 151–155 (1950)

219 Osgood E. E Monocytic leukemia report of 6 cases and review of 127 cases *Arch Int Med* 59 931 951 (1937)

220 Osgood E E Baker R. L Brownlee B A Osgood M W Ellis D M and Cohen W Total differential and absolute leukocyte counts and sedimentation rates of healthy children four to seven years of age *A M A Am J Dis Child* 58 61–70 (1939)

221 Ottander O Hochgradige Endotheliose im Blut bei Endocarditis lenta *Acta med Scandinav* 63 336–354 (1925–1926)

222 Owren P A Cyclic agranulocytosis *Acta med Scandinav* 134 87–97 (1949)

223 Owren P A Congenital hemolytic jaundice The pathogenesis of the hemolytic crisis *Blood* 3 231–248 (1948)

224 Pappenheim A *Clinical examination of the blood and its technique A manual for students and practitioners* (trans and adapt from German by R Donaldson Wood New York 1914)

225 Pappenheimer A M Thompson W P Parker D D and Smith K E Anaemia associated with unidentified erythrocyte inclusions after splenectomy *Quart J Med* 14 75–100 (1945)

226 Parsons W B Cooper T and Scheifly C H Anemia in bacterial endocarditis *J A M A* 153 14–16 (1953)

227 Patek A J Jr and Castle W B Plasma cell leukemia *Am J M Sc* 191 788–794 (1936)

228 Paul J R. and Bunnell W W The presence of heterophile antibodies in infectious mononucleosis *Am J M Sc* 183 90–104 (1932)

229 Pauling L Itano H A Singer S J and Wells I C "Sickle cell anemia a molecular disease *Science* 110 543–548 (1949)

230 Pearse A. G E Nature of Russell bodies and Kurloff bodies observations on cytochemistry of plasma cells and reticulum cells *J Clin Path* 2 81–90 (1949)

231 Pepper O H P The hematology of subacute streptococcus viridans endocarditis *J A M A* 89 1377–1380 (1927)

232 Pohle F J The blood platelet count in relation to the menstrual cycle in normal women *Am J M Sc* 197 40–47 (1939)

233 Powell W N Rodarte J G and Neel J V The occurrence in a family of Sicilian ancestry of the traits for both sickling and thalassemia *Blood* 5 887–897 (1950)

234 Price Jones C *Red blood cell diameters* (Oxford Medical Publications London 1933)

235 Ranney H M Observations on the inheritance of sickle-cell hemoglobin and hemoglobin C *J Clin Investigation* 33 1634–1641 (1954)

236 Rawson R Parker F Jr and Jackson H Jr Industrial solvents as possible etiologic agents in myeloid metaplasia *Science* 93 541 (1941)

237 Rebuck J W The structure of the giant cells in the blood forming organs *J Lab & Clin Med* 32 660–699 (1947)

238 Reich C A case of adenocarcinoma of the sigmoid with lymphatic leukemia blood picture *Am J Cancer* 26 781–782 (1936)

239 Reich C and Rumsey W Jr Agnogenic myeloid metaplasia of the spleen report of five cases illustrating diagnostic difficulties and danger of splenectomy and radiation therapy *J A M A* 118 1200–1204 (1942)

240 Reiman H A The blood platelets in pneumococcus infections *J Exper Med* 40 553–565 (1924)

241 Reichad H and Schilling Torgau V Ueber eine neue Leukämie durch echte Uebergangsformen (Splenozyten leukämie) und ihre Bedeutung für die Selbständigkeit dieser Zellen *München med Wchnschr* 60 1981–1989 (1913)

242 Reznikoff P White blood cell counts in convalescence from infectious diseases *Am J M Sc* 184 167–184 (1932)

243 Rich A Studies on the hemoglobin of Cooley s anemia and Cooley s trait *Proc Nat Acad Sc* 38 187–196 (1952)

244 Richter M N The presence of Auer bodies in leukemic tissues *Arch Int Med* 31 677–685 (1923)

245 Richter M N Observations on the hemohistioblast of Ferrata *Am J M Sc* 169 336–445 (1925)

246 Rigas D A Koler R D and Osgood E E New hemoglobin possessing higher electrophoretic mobility than normal adult hemoglobin *Science* 121 372 (1955)

247 Rolland C F and Davidson L S P A case of cyclic neutropenia *Edinburgh M J* 57 205–210 (1950)

248 Rous P and Robertson O H The normal fate of erythrocytes I The findings in healthy animals *J Exper Med* 25 651–663 (1917)

249 Rowley M W The occurrence of atypical phagocytic cells in the circulating blood *New York M J* 85 674–675 (1907)

250 Rowley M W A fatal anaemia with enormous numbers of circulating phagocytes *J Exper Med* 10 78–96 (1908)

251 Sabin F R and Doan C A The presence of desquamated endothelial cells the so-called clasmatocytes in normal mammalian blood *J Exper Med* 43 823–837 (1926)

252 Sabin F R Doan C A and Cunningham R S Discrimination of two types of phagocytic cell in the connective tissues by the supravital tech

nique *Carnegie Institution of Washington Pub No* 361 (1925) p 125 (Contributions to embryology No 82)

253 Sandoz *Atlas of haematology* (Sandoz Ltd Basle Switzerland and Sandoz Pharmaceutical New York 1952)

254 Schilling F V Ein praktisch und zur Demonstration brachbarer Differential leukozytometer mit Arnethscher Verschiebung des Blutbildes *Deutsch med Wchnschr* 37 1159–1163 (1911)

255 Schleicher E M The origin and nature of the Cabot ring bodies of erythrocytes *J Lab & Clin Med* 27 983–1000 (1942)

256 Schneider R G Incidence of hemoglobin C trait in 505 normal negroes a family with homozygous hemoglobin C and sickle cell trait union *J Lab & Clin Med* 44 133–144 (1954)

257 Schwartz S O and Motto S A The diagnostic significance of burr red blood cells *Am J M Sc* 218 563–566 (1949)

258 Schwartz H C Spaet T H Zuelzer W W, Neel J V Robinson A R and Kaufman S F Combinations of hemoglobin G hemoglobin S and thalassemia occurring in one family *Blood* 12 238–250 (1957)

259 Shen S C Fleming E M and Castle W B, Studies on the destruction of red blood cells V Irreversibly sickled erythrocytes their experimental production in vitro *Blood* 4 498–504 (1949)

260 Shen S C Ham T H and Fleming E M Studies on the destruction of red blood cells III Mechanism and complications of hemoglobinuria in patients with thermal burns spherocytosis and increased osmotic fragility of erythrocytes *New England J Med* 229 701–713 (1943)

261 Silvestroni E and Bianco I Genetic aspects of sickle cell anemia and microdrepanocytic disease *Blood* 7 429–435 (1952)

262 Singer K Hereditary hemolytic disorders associated with abnormal hemoglobins *Am J Med* 18 633–652 (1955)

263 Singer K Chapman A Z Goldberg S R Rubinstein H M and Rosenblum S A Studies on abnormal hemoglobins IX Pure (homozygous) hemoglobin C disease *Blood* 9 1023–1031 (1954)

264 Singer K Chernoff A I and Singer L Studies on abnormal hemoglobins I Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation *Blood* 6 413–435 (1951)

265 Singer K Fisher B and Perlstein M A Acanthrocytosis a genetic erythrocytic malformation *Blood* 7 577–591 (1952)

266 Singer K Josephson A M Singer L Heller P and Zimmerman H J Studies on abnormal hemoglobin XIII Hemoglobin S — thalassemia disease and hemoglobin C — thalassemia disease in siblings *Blood* 12 593–602 (1957)

267 Singer K Kraus A P Singer L Rubinstein H M and Goldberg S R Studies on abnormal hemoglobin X A new syndrome hemoglobin C — thalassemia disease *Blood* 9 1032–1046 (1954)

268 Singer K Miller E B and Dameshek W Hematologic changes following splenectomy in man with particular reference to target cells hemolytic index and lysolecithin *Am J M Sc* 202 171–187 (1941)

269 Singer K Singer I and Goldberg S R Studies on abnormal hemoglobins XI Sickle cell — thalassemia disease in the Negro the significance of the S + A + F and S + A patterns obtained by hemoglobin analysis *Blood* 10 405–415 (1955)

270 Smith C H Acute infectious lymphocytosis a specific infection report of four cases showing its communicability *J A M A* 125 342–349 (1944)

271 Smith E W and Conley C L Filter paper electrophoresis of human hemoglobins with special reference to the incidence and clinical significance of hemoglobin C *Bull Johns Hopkins Hosp* 93 94–106 (1953)

272 Smith E W and Conley C L Clinical features of the genetic variants of sickle cell disease *Bull Johns Hopkins Hosp* 94 289–318 (1954)

273 Smith E W and Conley C L Sicklemia and infarction of the spleen during aerial flight *Bull Johns Hopkins Hosp* 96 35–41 (1955)

274 Snell F M Neel J V and Ishibashi K Hematologic studies in Hiroshima and a control city two years after the atomic bombing *Arch Int Med* 84 569–604 (1949)

275 Spence H M and Roberts G M Extreme leukocytosis and acute hemolytic anemia associated with the administration of sulfanilamide *New England J Med* 222 874–876 (1940)

276 Statement concerning a system of nomenclature for the varieties of human hemoglobin *Blood* 8 386 (1953)

277 Stetson R P Normal variations in white blood cells under conditions of minimal metabolism *Arch Int Med* 40 488–495 (1927)

278 Stewart S G Familial eosinophilia *Am J M Sc* 185 21–29 (1933)

279 Strauss M B and Daland G A Hereditary ovalocytosis (human eliptical erythrocytes) Observations on ten cases in one family *New England J Med* 217 100–103 (1937)

280 Strumia M M *Hematological tables with brief notes* (Bryn Mawr Hospital Bryn Mawr Pa 1937)

281 Sturgeon P Itano H A and Bergren W R Clinical manifestations of inherited abnormal hemoglobins I The interaction of hemoglobin S with hemoglobin D *Blood* 10 389–396 (1955)

282 Sturgeon P Itano H A and Bergren W R Clinical manifestations of inherited abnormal hemoglobins II Interaction of hemoglobin E and thalassemia trait *Blood* 10 396–404 (1955)

283 Taliaferro W H and Kluver C The hematology

of malar a (plasmod um brasil anum) n Panamanian monkeys morphology of leucocytes and o igin of monocytes and macrophages *J I fect Dis* 67 162 1 6 (1940)

284 Terry D W Motulsky A G and Rath C E Homozygous hemoglobin C a new hereditary hemolyt c d ease *New E gland J Med* 251 365–373 (1954)

285 Thannhauser S J L po doses *Diseases of the cellular l pid metabolis n* (Oxford Med cine Ed Chr tian H A Oxford Un ers ty Press Inc New York 1949)

286 Thewl s E and M ddleton W S The leukocyt c p ctu e n catarrhal jaund ce (cholang t s) *Am J M Sc* 169 59–68 (1925)

287 Thompson W P Abnormal t es in the wh te blood cell response (leukemo d atyp cal leukemia and leukopen c blood p cture) *Am J M Sc* 182 334–35? (1931)

288 Thompson W P and Illyne C A Cl n cal and hematolog c p cture resulting from bone ma row eplacement *M Cl n North Amer ca* 24 841 853 (1940)

289 Thorn G W Forsham P H Prunty F T G and Hills A G The response to p tu tary adrenocort cotrop c hormone as a test for adrenal cort cal insufficiency *J A M A* 137 1005 1009 (1948)

290 Tileston W Fam lial sh ft to the left of the l u kocytes (Pelger s nuclear anomaly of the leukocytes) w th a report of a case *A n Int Med* 11 675–681 (1937)

291 Tocantins L M The mammal an blood platelet n health and d sease *Med cine* 17 155–260 (1938)

29? Valentine W N and Neel J V Hematolog c and genet c study of the transm ss on of thalassemia (Cooley s anem a Med terranean anemia) *Arch Int Med* 74 185–196 (1944)

293 Valent ne W N and Neel J V The art fic al p oduct on and s gn ficance of target cells w th special refe ence to the r occurrence n thalassem a (Cooley s erythroblast c anem a) *Am J M Sc* 209 741 752 (1945)

294 Vaughan J and Harr son C V Leucoerythroblast c anem a and myeloscl ros s *J Path & Ba t* 48 339–352 (1939)

295 Volin I F Greenspan I Ehrl ch L Gonner J A Fel enfeld O and Schwartz S O Hema topo et c changes dur ng adm n strat on of chloram phen col (chloromycet n) *J A M A* 142 1333 1335 (1950)

296 Watk ns C H and Hall B E Monocyt c l u kem a of the Naegel and Sch lling types *Am J Cl n Pa h* 10 387 396 (1940)

297 Watson C J The porphyr ns and d eases of the blood n *A sympos un on the blood a d blood fo m ng org ns* (Un vers ty of W scons n Press Mad on W s 1939) p 14

298 Webster S H He nz body phenomenon n e yth rocytes *Blood* 4 479–497 (1949)

299 Webster S H L ljegren E J and Z mmer D J He nz body fo mat on by certa n chem cal agents *J Pharma ol & Exp r The ap* 95 201 ?11 (1949)

300 Wh pple G H and Bradfo d W L Med ter ranean d sease thalassem a (erythroblast c ane m a of Cooley) *J Ped at* 9 279–313 (1936)

301 Wh tby L and Br tton C J C *D o ders of the blood D agno is pathology trea ment technique* (Church ll London ed 6 1950)

30? Wh te J C and Bea an G H A rev ew of the var et es of human haemoglob n n health and d sease *J Cl n Path* 7 175 00 (1954)

303 W ener H A and Morkovin D C rculating blood eos noph ls in acute nfect ous d sease and the eos nopen c response *An J Med* 13 58 72 (195?)

304 W lson C P and Cunningham R S A cons d erat on of the supravital method of studying blood n cases of mononuclear cell response *Folia haemat* 38 14–29 (19?9)

305 W ntrobe M M The size and hemoglob n con tent of the erythrocyte *J Lab & Cl n Med* 17 899–912 (193?)

306 Wintrobe M M *Cl n cal hematology* (Lea & Fe b ger Ph lade ph a ed 4 1956)

307 W ntrobe M M Matthews E Po lock R and Dobyns B M A fam l al hemopo et c d sorder in Italian a lole cents and adult resembl ng Med terranean disease (thalassem a) *J A M A* 114 1530–1538 (1940)

308 W seman B K Cr teria of the age of lymphocytes n the per pheral blood *J Exper Med* 54 271 294 (1931)

309 W seman B K and Doan C A Pr mary splen c neutropen a a newly recogn zed synd ome closely related to congen tal hemolyt c cterus and essent al thrombocytopen c purpura *An Int Med* 16 1097 1117 (1942)

310 W lock G B Bunt ng H and Dempsey E W Further ob e vat on on the chem cal cytology of megakaryocytes and other cell of hemopo et c t ssue *Anat Rec* 98 5?7 537 (1947)

311 Wr ght J H The or g n and nature of the blood plates *Bo ton M & S J* 154 643–645 (1906)

312 Wr ght J H The h stogenes s of the blood platelets *J Morphol* 21 263 ?78 (1910)

313 Wyandt H Bancroft P M and W nsh p T O Ell p c erythrocytes in man *Arch Int Med* 68 1043–1065 (1941)

314 Zinkham W H and D amond L K In vitro erythrophagocytos s n acqu ed hemolytic anem a *Blood* 7 592 601 (1952)

315 Zuelzer W W and Kaplan E Thalassem a—hemoglobin C disease a ne v syndrome presumably due to a comb nat on of genes for the thalassem a and hemoglob n C *Blood* 9 1047 1054 (1954)

316 Zuelzer W W Neel J V and Robinson A R Abnormal hemoglob ns in *Prog ess i hematology* e l Tocant ns L M (Grune and Stratton New York 1956) pp 91 137

INDEX

PLATE I TYPICAL CELLS OF THE RED CELL SERIES
(*Text on page 8*)

Key

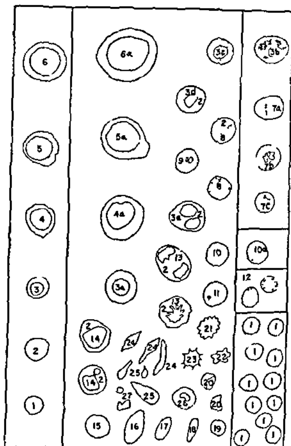
1 Normal red cell
2 Polychromatophilic cell
3 Normoblast
3a Normoblast — pathologic [a]
3b Normoblast with reticulum [b]
3c Normoblast with stippling
3d Normoblast with Howell Jolly bodies
3e Normoblast with double nucleus
4 Late erythroblast
4a Late erythroblast — pathologic
5 Early erythroblast
5a Early erythroblast — pathologic [a]
6 Proerythroblast
6a Proerythroblast — pathologic
7a Early reticulocyte [b]
7b Intermediate reticulocyte [b]
7c Late reticulocyte [b]
8 Stippled cell
9 Howell Jolly bodies
10 Siderocytes
10a Siderocyte stained for iron
11 Refractive granule
12 Heinz bodies [d]
13 Nucleated red cells showing mitosis
14 Cabot ring forms
15 Round macrocyte
16 Oval macrocyte
17 Oval cell
18 Pencil form
19 Spheroidal red cell
20 Lunar forms (partially hemolyzed)
21 Crenated cell
22 Burr cell
23 Acanthrocyte
24 Sickled forms
25 Pear shaped or tailed forms
26 Target cell
27 Irregular forms

These pathologic or abnormal cells are called megaloblasts by many authors

[b] Vitally stained with *brilliant cresyl blue before being* counterstained with Wright s stain

Stained with a solution of equal parts 1 percent hydrochloric acid and 2 percent potassium ferrocyanide and counterstained

[d] Vitally stained with saturated solution of methyl violet in 0 73 percent sodium chloride



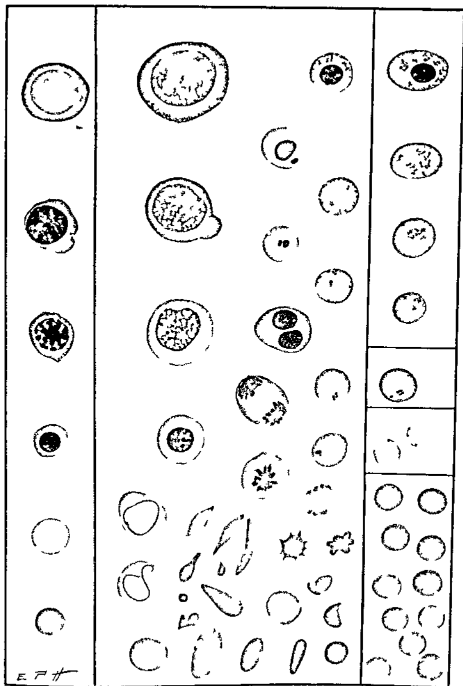


PLATE I. TYPICAL CELLS OF THE RED CELL SERIES

PLATE II TYPICAL CELLS OF NORMAL HUMAN BLOOD
(Text on page 16)

Key
1 Normal red cells
2 Platelets
3 Neutrophil adult
3a Neutrophil adult (two lobes)
4 Neutrophil band form
5a Eosinophil two lobes
5b Eosinophil band form
6a Basophil band form
6b Metamyelocyte basophilic
7 Lymphocyte small
8 Lymphocyte large
9 Monocyte mature
10 Monocyte young

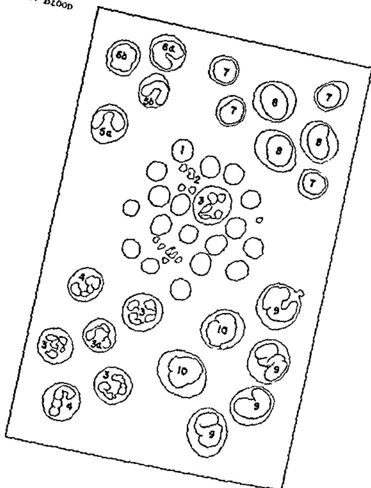


TABLE 4 MEAN VALUES OF WHITE CELL COUNTS AND DIFFERENTIAL WHITE CELL COUNTS IN TWENTY NORMAL ADULTS *

	Ear lobe		Fingertip		Antecubital vein	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
White cell count (10³/mm.)	8.03	1.58	6.[illegible]7	1.37	6.66	1.42
Differential white-cell count (percent)						
Neutrophils adult	49.65	8.40	47.22	6.47	47.90	[illegible].26
Neutrophil band	12.[illegible]0	5.81	12.97	5.37	12.5[illegible]	5.62
Eosinophils	3.1[illegible]	2.19	2.30	1.66	2.57	1.59
Basophils	0.72	0.72	0.65	0.84	0.4[illegible]	1.64
Metamyelocytes	0.10	—	0	—	0	—
Lymphocytes small	21.5[illegible]	7.95	25.40	5.74	23.80	6.2[illegible]
Lymphocytes large	3.0[illegible]	2.16	2.50	2.53	4.00	1.51
Lymphocytes young and atypical	0.60	—	0.45	—	1.27	—
Monocytes	7.[illegible]0	3.94	7.95	2.58	7.05	3.3[illegible]
Plasma cell	0.0[illegible]	—	0.0	—	0.05	—
Histiocytes	0.35	—	0.3	—	0.35	—

Blood samples were taken simultaneously from different sites. In each individual the white cells were counted using 2 pipettes and 2 chambers counting an area of 8 square millimeters for each pipette. The differential white-cell counts were the result of counting 100 cells on each of two films. The first drop was discarded, the second drop was used for white-cell counts and the third for the coverslip preparations of the blood films.

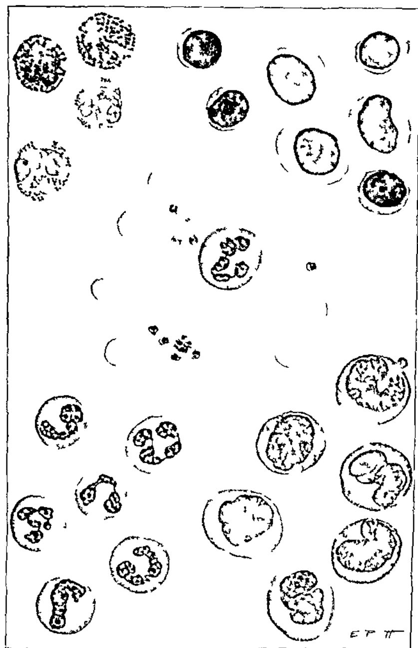


Plate III Pernicious Anemia
(Text on page 3)

Upper picture Before treatment (Wright's stain)
Lower picture Six days after initial treatment with liver extract showing reticulocyte (stained with brilliant cresyl blue and counterstained with Wright's stain)

Key
1 Neutrophil
1a Multilobed neutrophil
2 Lymphocyte
3 Normal red cell
4 Normoblast with reticulum
5 Late erythroblast with reticulum
6a Early reticulocyte
6b Intermediate reticulocyte
6c Late reticulocyte
7 Stippled cell
8 Polychromatophilic cell
9 Oval macrocyte
10 Round macrocyte
11 Microcyte
12 Tailed or pear shaped forms
13 Irregular forms
14 Platelets

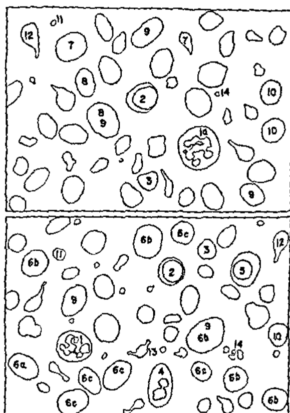


Table 5 Laboratory Data for Plate III Pernicious Anemia

Observations	Before treatment (upper picture)	Six days after initial treatment (lower picture)
Red cell count (10 mm)	1 16	1 2
Hemoglobin (gm/100 ml)	4 6	4
Hematocrit (percent)	14	1 8
Red cell indices		
MCV [mean corpuscular volume (μ)]	12	127
MCHC [mean corpuscular hemoglobin concentration (gm/100 ml of cells)]	32	2)
MCH [mean corpuscular hemoglobin ($\mu\mu$g)]	3)	38
Reticulocytes (percent)	1 0	21
White cell count (10 mm³)	?0	5 4
Platelet count (10 /mm³)	)5 0	210 0
Icterus index (units)	1	5

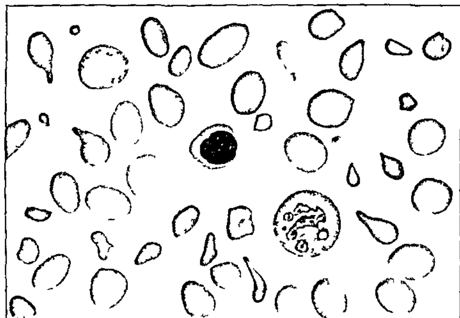




PLATE III. PERNICIOUS ANEMIA

PLATE I\ H\POCHROMIC A\EMIA
(*Text on page 34*)

Lpper p ct re Moderate h pochrom c anemia in a 17 year old g rl before treatment

Lo.er p ct re Se ere hypochrom c anemia from chron c blood loss (hemorrho ds) n a 6 year old man before treat ment

Ke

1 \eu oph l
2 Small l mphocyte
3 Monoc te
4 \ormal red cell
5 Macro te
6 M croc te
7 Penc l form
8 Target form
9 Pol chromatoph l c cell
10 St ppled m crocyte
11 \ormobla t
12 Irregular forms
13 Platelets

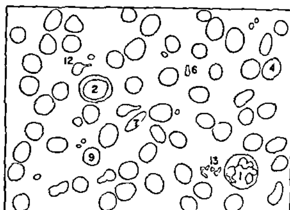
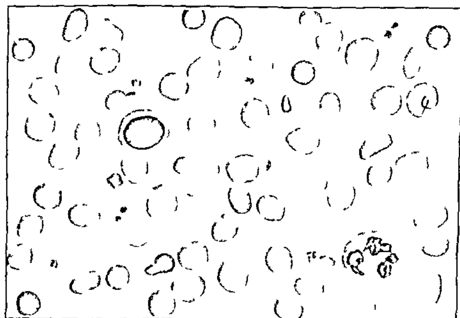




TABLE 6 I ABORATOR\ DATA FOR PLATE I\
H\PO HROM C A\EMIA

Ob er at ons	Upper p ctu e	Lo er p cture
Red-cell count (10 mm)	5 1	1 2
Hemoglob n (gm 100 ml)	9 2	2 0
Hematocr t (percent)	32 0	7 8
Red-cell nd ces		
MC\ [mean corpuscular volume (μ)]	6?	65
MCHC [mean orpuscular hemoglob n concentrat on (gm 100 ml of cell)]	29	26
MCH [mean corpuscular hemoglob n μμg]	18	1
Ret cul cy e pe cent)	?	2
Platelet count (10^3 mm^3)	250	?00
Wh e-cell count ($10^3/mm^3$)	10 6	7 6
Icterus ndex (un ts)	4	?



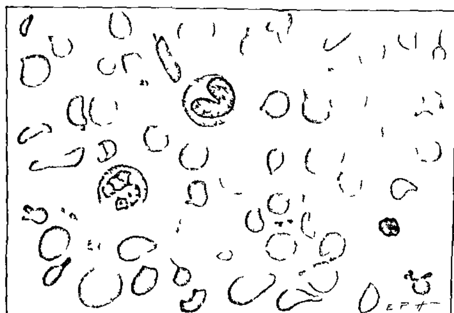


PLATE IV HYPOCHROMIC ANEMIA

PLATE V COOLEY'S ANEMIA
(*Text on page 35*)

Key
1 Neutrophil
2 Young lymphocyte
3 Myelocyte
4 Normal red cell
5 Macrocyte
6 Microcyte
7 Pencil form
8 Target form
9 Polychromatophilic red cell
10 Stippled cell
11 Cabot ring form
12 Red cell with Howell Jolly body
13 Normoblast
14 Late erythroblast
15 Early erythroblast
16 Irregular forms
17 Platelets

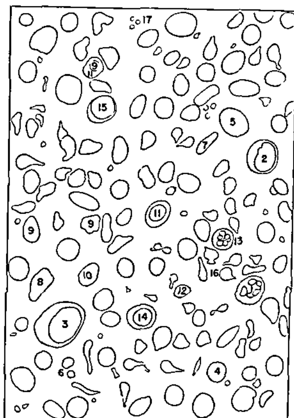


TABLE 7 LABORATORY DATA FOR PLATE V COOLEY'S ANEMIA

Red-cell count (10^6/mm³)	3 3
Hemoglobin (gm/100 ml)	6
Hematocrit (percent)	23 4
Red-cell indices	
MCV [mean corpuscular volume (μ^3)]	71
MCHC [mean corpuscular hemoglobin concentration (gm/100 ml of cells)]	28
MCH [mean corpuscular hemoglobin ($\mu\mu$g)]	20
Reticulocytes (percent)	6 6
White-cell count (10^3/mm³)	4 4
Icterus index (units)	10 0

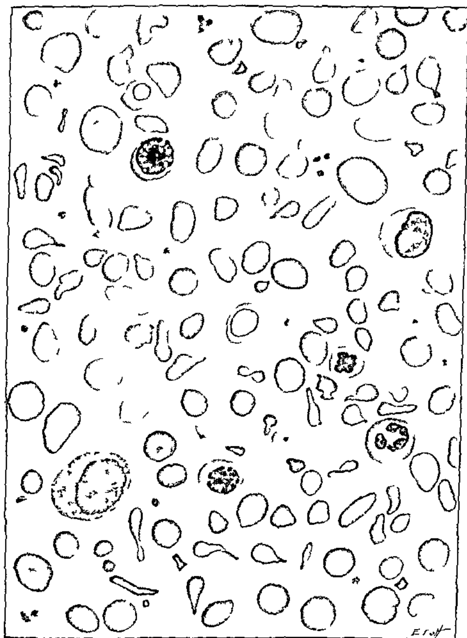


PLATE V. COOLEY ANEMIA

Plate VI Sickle Cell Anemia

(*Text on page 36*)

Upper picture Unmanipulated film of peripheral blood from a Negro girl

Lower picture Film of reduced (anoxic) venous blood from a Negro male

Key
1 Neutrophil
2 Lymphocyte
3 *Normal red cell*
4 Macrocyte
5 Polychromatophilic cell
6 Target form
Sickled cells
8 Normoblast
9 Late erythroblast
10 Platelets

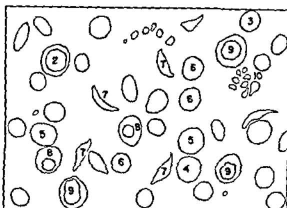




Table 8 Laboratory Data for Plate VI Sickle Cell Anemia

Observations	Upper picture	Lower picture
Red-cell count (10^6 mm³)	2 9	4 3
Hemoglobin (gm/100 ml)	7 9	9 4
Hematocrit (percent)	23 4	30 5
Red-cell indices		
MCV [mean corpuscular volume (μ^3)]	81	71
MCHC [mean corpuscular hemoglobin concentration (gm 100 ml of cells)]	34	31
MCH [mean corpuscular hemoglobin ($\mu\mu g$)]	27	22
Reticulocytes (percent)	6	3
Nucleated red cells (per 100 white cells)	5	4
White-cell count (10^3 mm³)	17	14 5
Icterus index (units)	1	17

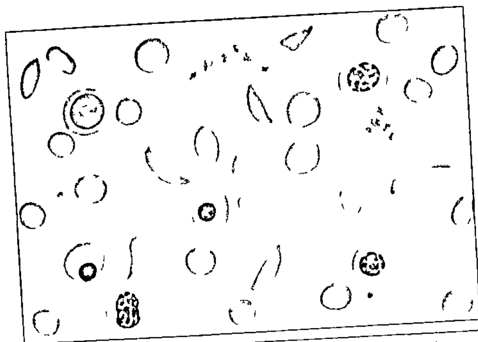




PLATE VI SICKLE CELL ANEMIA

PLATE VII HEMOLYTIC ANEMIA — CHRONIC AND ACUTE

(*Text on page 38*)

Upper pictures Chronic hemolytic jaundice — Hereditary spherocytosis (two films from the same sample of blood *left* Wright's stain *right* stained with brilliant cresyl blue and Wright's stain)

Lower picture Acute hemolytic anemia following sulfanilamide therapy (Wright's stain)

Key

2 Young neutrophil (band)
3 Metamyelocyte
4 Myelocyte
5 Eosinophil
6 Monocyte
Small lymphocyte
8 Normal red cell
9 Spherocyte
10 Polychromatophilic cell
11 Reticulocyte
12 Macrocyte
13 Irregular red cell (lunar)
14 Normoblast
15 Late erythroblast
16 Platelets

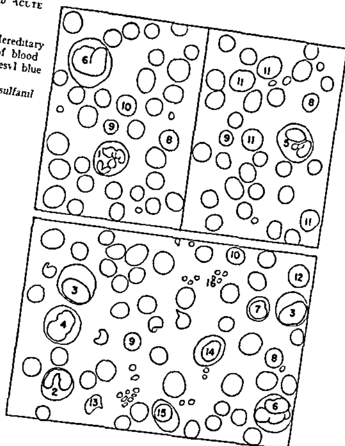


TABLE 9 LABORATORY DATA FOR PLATE VII HEMOLYTIC ANEMIA

Observations	Chronic hemolytic jaundice (upper pictures)	Acute hemolytic anemia (lower picture)
Red-cell count (10^6/mm)	4 5	1 4
Hemoglobin (gm/100 ml)	12 0	4 6
Hematocrit (percent)	34 6	15 5
Red cell indices		
MCV [mean corpuscular volume (μ^3)]	77	111
MCHC [mean corpuscular hemoglobin concentration (gm/100 ml of cells)]	35	30
MCH [mean corpuscular hemoglobin ($\mu\mu$)]	26	33
Reticulocytes (percent)	10 9	8 4
Osmotic fragility of red cells	Moderate increase	Marked increase
Spherocytes in blood film	Present	Present
White-cell count (10^3/mm^3)	11 6	54 0
Plasma bilirubin (mg/100 ml)	4 0	2 7
Plasma hemoglobin (mg/100 ml)	—	160
Hemoglobinuria	0	Marked

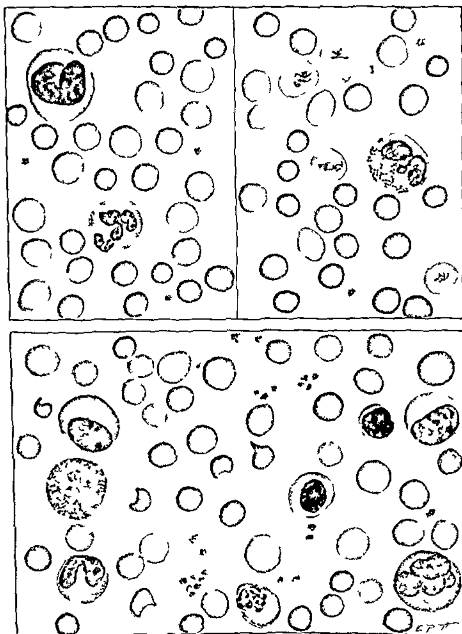


PLATE VII HEMOLYTIC ANEMIA CHRONIC AND ACUTE

PLATE VIII ERYTHROBLASTOSIS FETALIS

(Text on page 40)

Key

1 *Neutrophil*
2 Young neutrophil (band)
3 Small lymphocyte
4 Myelocyte
5 *Normal red cell*
6 Macrocyte
Microcyte
8 Polychromatophilic cell
9 Stippled cell
10 Howell Jolly bodies
11 Normoblast
12 Late erythroblast
12a Late erythroblast with double nucleus
13 Early erythroblast
14 Erythroblast with nucleus in mitosis
15 Proerythroblast
16 Platelets

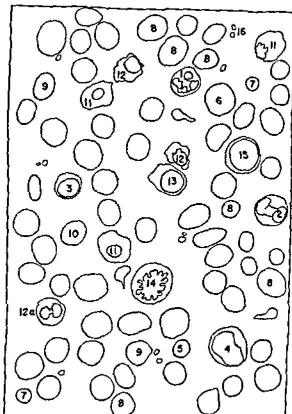


TABLE 10 LABORATORY DATA ON ONE NORMAL BABY AT BIRTH AND THREE CASES OF ERYTHROBLASTOSIS FETALIS

(Data are not given for the blood film in Plate VIII)

Observations	Normal baby at birth	Erythroblastosis fetalis		
		Baby D 10 hr	Baby T 48 hr	Baby K 10 hr
Red-cell count (10^6/mm)	4 8	4 6	4 4	3 0
Hemoglobin (gm/100 ml)	17 0	15 6	13 3	13 0
Hematocrit (percent)	53 5	53 1	40	43 4
Red cell indices				
MCV [mean corpuscular volume (μ^3)]	110	114	91	145
MCHC [mean corpuscular hemoglobin concentration (gm/100 ml of cells)]	32	29	33	30
MCH [mean corpuscular hemoglobin ($\mu\mu$g)]	35	34	30	43
Reticulocytes *(percent)*	1 9	6 3	11	21
Nucleated red cells (per 100 white cells)	1	11	3	53 *
(per mm³)		2134		48 000
Icterus index (units)	—	30	200+	?25
White-cell count (10^3/mm³)				
Uncorrected for nucleated red cells	9 6	19 4	8 4	54
Differential white count				
Neutrophils adult	38	54	54	16
Neutrophils band	5	23	21	3?
Eosinophils	2	1	1	1
Basophils			1	
Metamyelocytes		3		4
Myelocytes	2	2	6	13
Myeloblasts				20
Lymphocytes small	31	6	8	6
Lymphocytes large	1	4	3	1
Lymphocytes young			1	
Lymphocytes atypical		1		
Monocytes adult	14	6	5	6
Monocytes young	7			
Histiocytes				1

* The nucleated red cells seen while counting 200 white cells included 40 normoblasts, 12 erythroblasts, and 1 proerythroblast

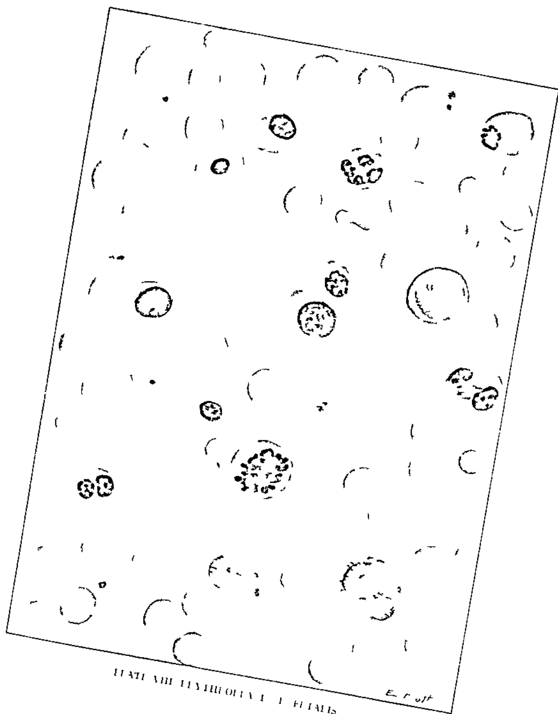


PLATE VIII. ERYTHROBLASTOSIS FETALIS

PLATE X LEUKOCYTOSIS
(*Text on page 41*)

Key
1 Neutrophil adult
2 Young neutrophil (band)
2a Young neutrophil with Dohle body
3 Neutrophilic metamyelocyte
4 Neutrophilic myelocyte C
5 Neutrophilic myelocyte B
6 Small lymphocyte
7 Large lymphocyte
8 Monocyte
9 Histiocyte
10 Plasma cell
11 Red cell
12 Platelet

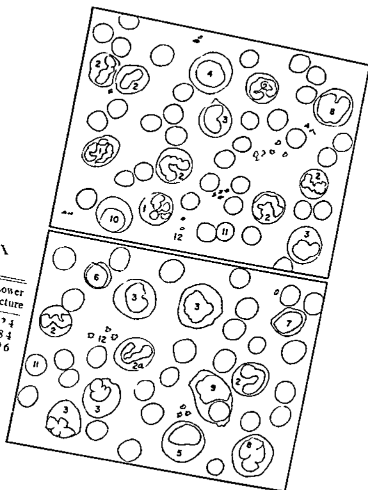
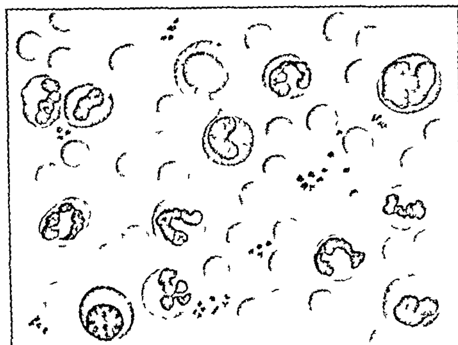


TABLE 12 LABORATORY DATA FOR PLATE X LEUCOCYTOSIS

Observations	Upper picture	Lower picture
Red cell count (10^6/mm³)	?6	?4
Hemoglobin (gm/100 ml)	6 9	8 4
Hematocrit (percent)	22	29 6
Red-cell indices		
MCV [mean corpuscular volume (μ^3)]	84	122
MCHC [mean corpuscular hemoglobin concentration (gm 100 ml of red cells)]	31	28
MCH [mean corpuscular hemoglobin $\mu\mu g$)]	77	35
White cell count (10 mm)	35 0	29 0
Differential white-cell count		
Neutrophils adult	40	20
Neutrophil band	46	54
Metamyelocyte	2	15
Myelocyte	1	3
Lymphocytes small	6	3
Lymphocytes large		1
Monocytes	4	3
Histiocytes		1
Plasma cell	1	



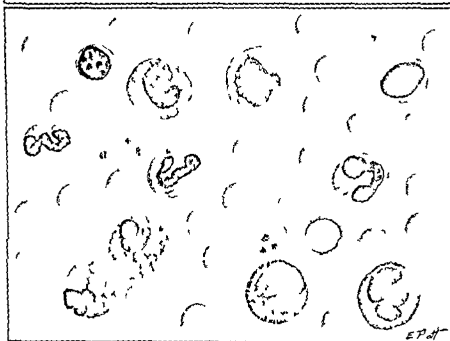


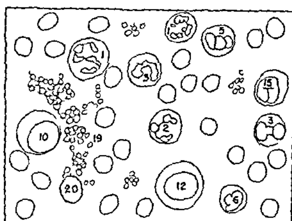
PLATE X LEUCOCYTOSIS

PLATE XI MYELOGENOUS LEUKEMIA

(*Text on page 44*)

Key

1 Neutrophil
2 Eosinophil
3 Young neutrophil (band)
4 Young eosinophil
5 Young basophil
6 Abnormal basophil
7 Neutrophilic metamyelocyte
8 Neutrophilic myelocyte C or late myelocyte
9 Eosinophilic myelocyte C
10 Neutrophilic myelocyte B
11 Eosinophilic myelocyte B
12 Basophilic myelocyte B
13 Myelocyte A or early myelocyte
14 Myeloblast
15 Young monocyte
16 Normal red cell
17 Normoblast
18 Late erythroblast
19 Platelets
20 Megakaryocyte nucleus



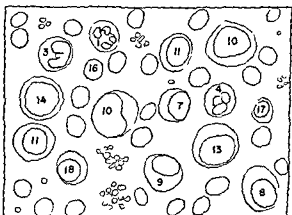


TABLE 13 LABORATORY DATA FOR PLATE XI MYELOGENOUS LEUKEMIA

Observations	Upper picture	Lower picture
Red cell count (10^6 mm³)	3.5	2.4
Hemoglobin (gm/100 ml)	9.2	7.6
Hematocrit (percent)	29.8	27.6
Red-cell indices		
MCV [mean corpuscular volume (μ^3)]	85	95
MCHC [mean corpuscular hemoglobin concentration (gm/100 ml of cells)]	31	34
MCH [mean corpuscular hemoglobin ($\mu\mu g$)]	26	31
Reticulocytes (percent)	2	4
Nucleated red cells (per 100 white cells)	1	5
Platelet count (10^3 mm³)	300	250
White cell count (10^3/mm³)	50	94
Differential white-cell count (percent)		
Neutrophils adult	27	15
Neutrophil band	15	16
Eosinophils	2	3
Basophils	10	2
Metamyelocytes	2	11
Myelocytes C neutrophilic	5	11
Myelocytes C eosinophilic	1	4
Myelocytes B *neutrophilic*	24	19
Myelocytes B eosinophilic	1	4
Myelocytes B basophilic	5	2
Myelocytes A		3
Myeloblasts		7
Lymphocytes	5	2
Monocytes	3	1

PLATE XI MYELOGENOUS LEUKEMIA

PLATE XII MONOCYTIC LEUKEMIA

(*Text on page 4*)

Key

1 Neutrophil
2 Young neutrophil (band)
3 Small lymphocyte
4 Neutrophilic myelocyte B
5 Histiocyte
6 Monocyte adult
Monocyte young
8 Monoblast
8a Monoblast showing definite nucleoli
8b Monoblasts with Auer bodies
8c Monoblasts with azurophilic granulation
9 Normal red cells
10 Platelets

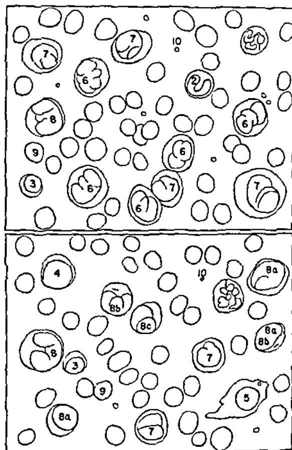


TABLE 14 LABORATORY DATA FOR PLATE XII MONOCYTIC LEUKEMIA

Observations	Upper picture	Lower picture
Red-cell count (10⁶ mm)	1 4	1 8
Hemoglobin (gm/100 ml)	5 3	6 0
Hematocrit (percent)	15 8	18 7
Red-cell indices		
MCV [mean corpuscular volume (μ]	111	104
MCHC [mean corpuscular hemoglobin concentration (gm 100 ml of cells)]	34	32
MCH [mean corpuscular hemoglobin μμg)	37	33
Reticulocytes (percent)	1 1	1 5
Nucleated red cells (per 100 white cells)	1	[illegible]
Icterus index (units)		5
Platelet count (10³/mm)	146	90
White-cell count (10³ mm³)	43	76
Differential white-cell count (percent)		
Neutrophil adult	9	10
Neutrophils band	14	[illegible]
Eosinophils	1	
Basophil		
Metamyelocytes		1
Myelocytes		2
Lymphocytes	[illegible]	16
Monocytes adult	40	20
Monocytes young	[illegible]	21
Monoblasts	4	23
Histiocyte		[illegible]

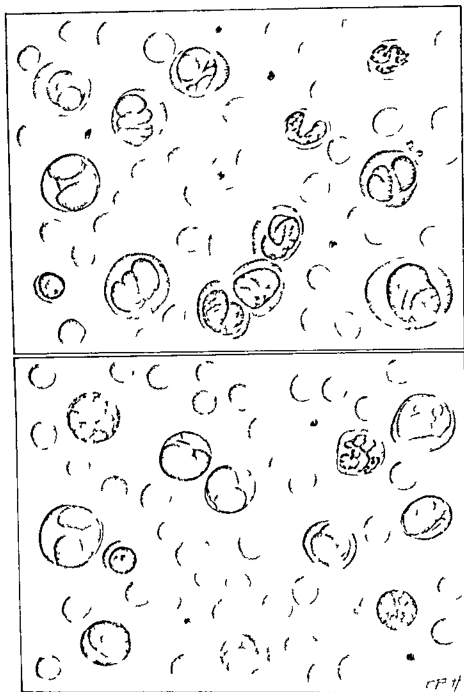


PLATE XII MONOCYTIC LEUKEMIA

PLATE XIII HISTIOCYTES

(*Text on page 50*)

Upper picture Bacterial endocarditis
Lower picture Histiocytic leukemia

Key

1 Neutrophil
2 Young neutrophil (band)
2a Young neutrophil with vacuoles
3 Small lymphocyte
4 Large lymphocyte
5 Monocyte
6 Histiocyte phagocytic ameboid or reactive type
7 Histiocyte basophilic or leukemic type
8 Histiocyte showing phagocytosis of red cells
9 Basket cell
10 Red cells
11 Platelets

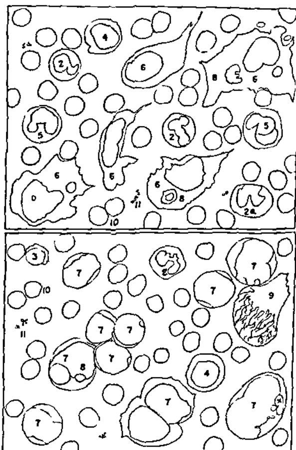


TABLE 15 LABORATORY DATA FOR PLATE XIII BACTERIAL ENDOCARDITIS AND HISTIOCYTIC LEUKEMIA

Observations	Bacterial endocarditis Upper picture	Histiocytic leukemia Lower picture
Red-cell count (10⁶/mm³)	3.8	1.6
Hemoglobin (gm/100 ml)	10.9	5
Hematocrit (percent)	34	15
Red-cell indices		
MCV [mean corpuscular volume (μ^3)]	89	94
MCHC [mean corpuscular hemoglobin concentration (gm/100 ml of cells)]	32	35
MCH [mean corpuscular hemoglobin ($\mu\mu g$)]	28	33
Reticulocytes (percent)	*1.0*	
Icterus index (units)	5	5
Platelet count ($10^3/mm^3$)		24.0
White-cell count ($10^3/mm^3$) *	88.0	28.9
Differential white-cell count (per cent)		
Neutrophil adult	21	
Neutrophils band	36	2
Lymphocytes small	10	10
Lymphocytes large	7	1
Monocytes	2	
Plasma cells	1	
Histiocytes	23	87

* White cell count and differential were done on blood from the ear lobe; the red-cell studies were determined on venous blood

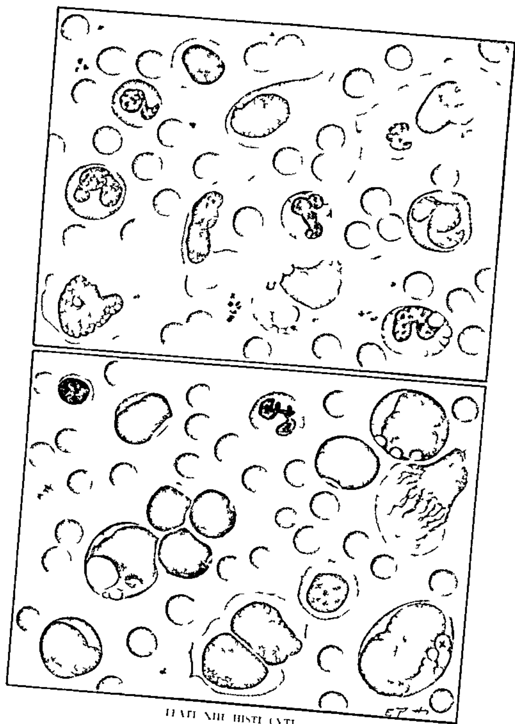


PLATE XIII HISTI CYTI

Plate XII Lymphocytic Leukemia

(Text on page 53)

Upper picture Chronic lymphocytic leukemia
Lower picture Acute lymphocytic leukemia

Key

1 Neutrophil
2 Monocyte
3 Small lymphocyte
4 Large lymphocyte
5 Young lymphocyte
6 Lymphoblast
7 Broken cell or smudge
8 Normal red cell
9 Polychromatophilic red cell
10 Platelets

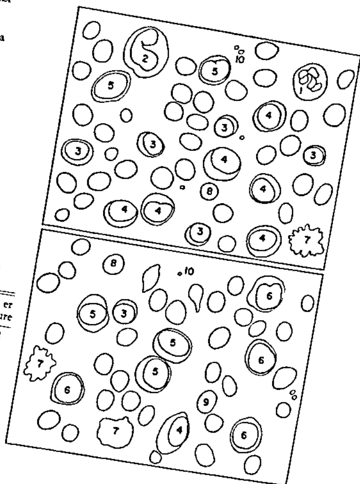
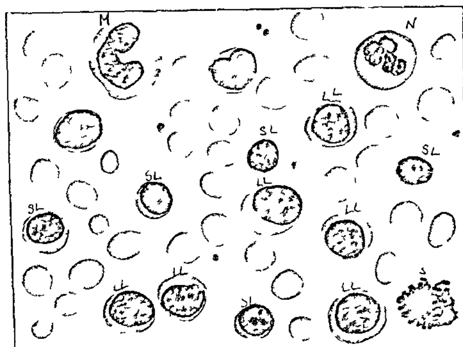


Table 16 Laboratory Data for Plate XII Lymphocytic Leukemia

Observations	Upper picture	Lower picture
Red-cell count (10^6/mm³)	3.8	2.1
Hemoglobin (gm/100 ml)	10.6	6.5
Hematocrit (percent)	36.4	19.[illegible]
Red-cell indices		
MCV [mean corpuscular volume (μ^3)]	96	92
MCHC [mean corpuscular hemoglobin concentration (gm/100 ml of cells)]	29	35
MCH [mean corpuscular hemoglobin ($\mu\mu g$)]	28	32
Reticulocytes (percent)	0.8	—
Icterus index (units)	4.0	—
White-cell count (10^3/mm³)	50.1	11.1
Differential white-cell count (percent)		
Neutrophils, adult	5	5
Neutrophils, band		
Eosinophils		
Basophils		
Myelocytes		3
Lymphocytes, small	83	12
Lymphocytes, large	10	5
Lymphocytes, young		10
Lymphoblasts		65
Monocyte	2	



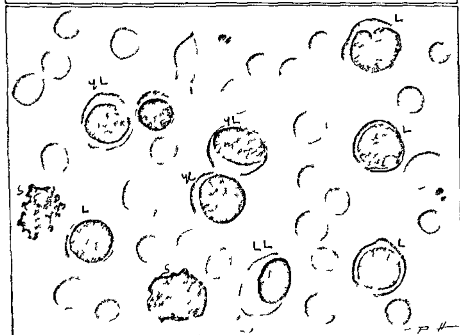


PLATE XIV LYMPHOCYTIC LEUKEMIA

Plate XX Plasma Cell Leukemia

(Text on page 54)

Key

1 Neutrophil
2 Small lymphocyte
3 Large lymphocyte
4 Monocyte
5 Mature plasma cell
5a Plasma cell with two nuclei
5b Plasma cell showing irregular periphery and purple color to cytoplasm
5c Plasma cell with Russell bodies
6 Young plasma cell
Plasmablast
8 Stem cell
9 Normal red cell
9a Red cell in rouleau
10 Normoblast
11 Late erythroblast
12 Platelets

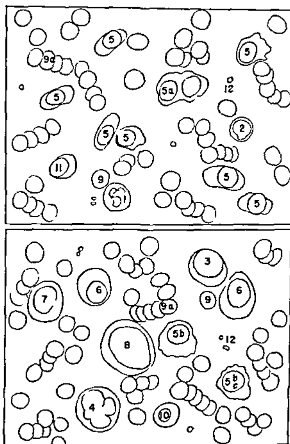
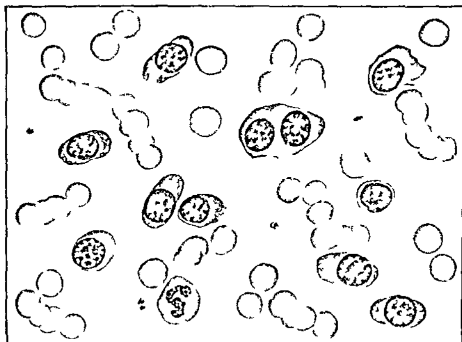


Table 17 Laboratory Data for Plate XX Plasma Cell Leukemia

Observations	Upper picture	Lower picture
Red-cell count ($10^6/mm^3$)	2 3	3 7
Hemoglobin (gm/100 ml)	8 7	10 1
Hematocrit (percent)	25 2	33 1
Red-cell indices		
MCV [mean corpuscular volume (μ^3)]	108	89
MCHC [mean corpuscular hemoglobin concentration (gm/100 ml of cells)]	34	31
MCH [mean corpuscular hemoglobin ($\mu\mu g$)]	37	27
Reticulocytes (percent)	—	1 5
Icterus index (units)	5	4
White-cell count ($10^3/mm^3$)	40 0	24 6
Differential white-cell count (percent)		
Neutrophils adult	6	26
Neutrophils band	21	15
Eosinophils		1
Basophils		
Myelocytes		3
Lymphocytes small	1	5
Lymphocytes large		5
Lymphocytes young		2
Monocytes	1	3
Plasma cell adult	32	20
Plasma cells young	39	15
Plasmablasts		5



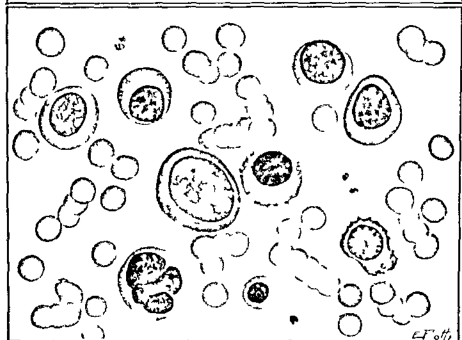


PLATE XX. PLASMA CELL LEUKEMIA

PLATE XVI INFECTIOUS MONONUCLEOSIS

(*Text on page 55*)

Key
1 Neutrophil band
2 Small lymphocyte
3 Monocyte
4 Atypical lymphocyte irregular type
5 Atypical lymphocyte vacuolated type
6 Atypical lymphocyte young type
7 Blast form probably lymphoblast
8 Plasma cell
9 Normal red cell
10 Platelets

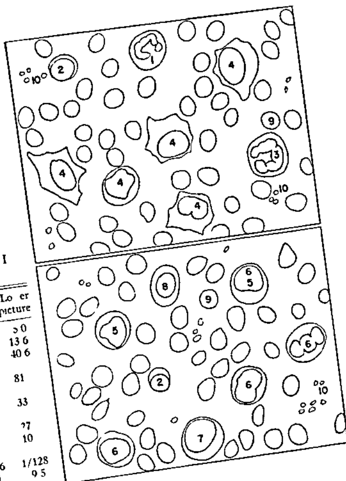


TABLE 18 LABORATORY DATA FOR PLATE XVI INFECTIOUS MONONUCLEOSIS

Observations	Upper picture	Lower picture
Red-cell count (10⁶ mm³)	4 1	5 0
Hemoglobin (gm/100 ml)	12 3	13 6
Hematocrit (percent)	39 1	40 6
Red-cell indices		
MCV [mean corpuscular volume (μ^3)]	96	81
MCHC [mean corpuscular hemoglobin concentration (gm/100 ml of cells)]	32	33
MCH [mean corpuscular hemoglobin ($\mu\mu g$)]	30	27
Icterus index (units)	4	10
Heterophile antibody (dilution showing agglutination)	1/256	1/128
White-cell count ($10^3/m^3$)	22 1	9 5
Differential white-cell count (percent)		
Neutrophils adult	27	7
Neutrophils band	9	8
Eosinophils		1
Basophils	1	
Lymphocytes small	4	32
Lymphocytes large	23	10
Lymphocytes young	7	18
Lymphocytes atypical	18	14
Lymphoblasts		2
Monocytes adult	8	6
Monocytes young	3	
Plasma cells adult		2

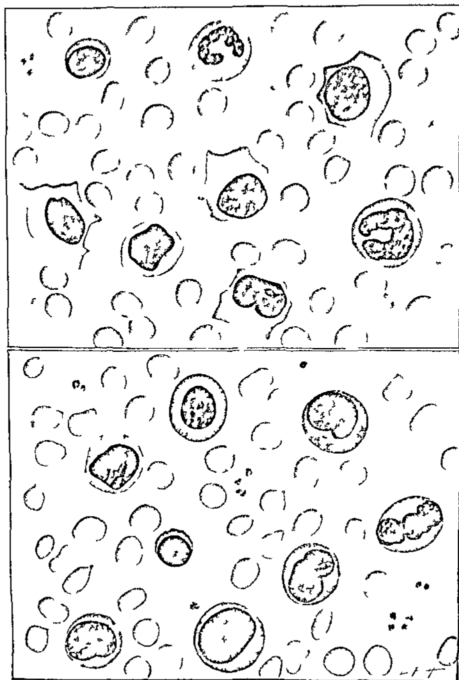


PLATE XVI INFECTIOUS MONONUCLEOSIS